

ARCHIVES OF PATHOLOGY

EDITORIAL BOARD

LUDVIG HEKTOEN, Chicago, Chief Editor

JAMES EWING, New York

OSCAR T. SCHULTZ, Evanston, Ill.

S. B. WOLBACH, Boston

GEORGE H. WHIPPLE, Rochester, N. Y.

FRANK R. MENNE, Portland, Ore.

Volume 30
1940

PUBLISHERS
AMERICAN MEDICAL ASSOCIATION
CHICAGO, ILL.

100

Dental Lib,
Direct Trfd to
Library
4/12/46

CONTENTS OF VOLUME 30

WOLBACH NUMBER

JULY 1940. NUMBER 1

	PAGE
Foreword. Walter B. Cannon, M.D., Boston.....	1
Rheumatic Disease of the Tricuspid Valve. Mark D. Altschule, M.D., and Edward Budnitz, M.D., Boston.....	7
Renal Lesions Associated with Deep Jaundice, with Comments on Their Relations to Those in the So-Called Hepatorenal Syndrome and in Transfusion Reactions. Darrell Ayer, M.D., Boston.....	26
Histologic Sequences in the Meningioma, with a Consideration of the Nature of Hyperostosis Cranii. Orville T. Bailey, M.D., Boston.....	42
Subcutaneous Nodules of Rheumatoid Arthritis and Rheumatic Fever: A Pathologic Study. Granville A. Bennett, M.D.; J. Wallace Zeller, M.D., and Walter Bauer, M.D., Boston.....	70
Rate of Dentin Formation in Incisor Teeth of Guinea Pigs on Normal and on Ascorbic Acid-Deficient Diets. Paul E. Boyle, D.M.D.; Otto A. Bessey, Ph.D., and Percy R. Howe, D.D.S., Boston.....	90
Osteoporosis Associated with Extensive Metastatic Calcification and Chronic Renal Disease. Charles L. Brown, M.D., and I. W. Ginsburg, M.D., Philadelphia	108
Effects of an Anterior Callosal Glioblastoma Multiforme on the Entire Brain. Myrtelle M. Canavan, M.D., Boston.....	122
✓ Healed Pulmonary Infarcts. Benjamin Castleman, M.D., Boston.....	130
Myoepithelial Hamartoma of the Gastrointestinal Tract: A Report of Eight Cases with Comment Concerning Genesis and Nomenclature. B. Earl Clarke, M.D., Providence, R. I.....	143
Distribution of Affected Nerve Cells in Amyotonia Congenita (Second Case). J. LeRoy Conel, Ph.D., Boston.....	153
Some Effects of Chronic Alcohol Poisoning in Rabbits. Charles L. Connor, M.D., San Francisco.....	165
Neuropathic Pulmonary Edema: Further Observations. Sidney Farber, M.D., Boston	180
Meningioma. Nathan Chandler Foot, M.D., New York.....	198
Immunity to Fowlpox Studied by Means of Skin Grafts on Chorioallantois of Chick Embryo. E. W. Goodpasture, M.D., and Katherine Anderson, Ph.D., Nashville, Tenn.....	212
Lead Absorption and Intoxication in Man Unassociated with Occupations or Industrial Hazards: Absorption of Lead from Eleven Weeks of Intra-uterine Life to Ninety-Three Years of Age. G. H. Hansmann, M.D., and M. C. Perry, M.A., Milwaukee.....	226
Amyloid: I. Methods of Isolating Amyloid from Other Tissue Elements. George Hass, M.D., and R. Z. Schulz, M.D., New York.....	240
Genesis of Hydatidiform Mole. Arthur T. Hertig, M.D., and Henry W. Edmonds, M.D., Boston.....	260
Visceral Lesions Associated with Varicella. Harald N. Johnson, M.D., Montgomery, Ala.	292
Mouse Leprosy. Cecil Krakower, M.D., and Luis M. González, B.S., San Juan, Puerto Rico.....	308
Pathology of Acute and of Healed Experimental Pyelonephritis. G. K. Mallory, M.D.; A. R. Crane, M.D., and J. E. Edwards, M.D., Boston...	330
Carcinoma in Situ of the Stomach and Its Bearing on the Histogenesis of Malignant Ulcers. Tracy B. Mallory, M.D., Boston.....	348
Mechanism of Leukocytosis with Inflammation: The Nature of the Leukocytosis-Promoting Factor in Exudates. Valy Mehkin, M.D., Boston	363
Toxoplasma Infection in Man. Henry Pinkerton, M.D., and David Weinman, M.D., St. Louis.....	374
Primary Carcinoma in the Negro: Anatomic Distribution of Three Hundred Cases. William S. Quinland, M.D., and J. R. Cuff, M.D., Nashville, Tenn.	393
Relation of Anatomic Pattern to Pathologic Conditions of the Coronary Arteries. Monroe J. Schlesinger, M.D., Ph.D., Boston.....	403
Hypersensitivity in the Isolated Rabbit Heart Following Intrapericardial Sensitization. Beatrice Carrier Seegal, M.D., and Herbert B. Wilcox Jr., M.D., New York.....	416
Pathologic Changes Observed in Human Tissues Subjected to Subcritical Temperatures. Lawrence W. Smith, M.D., Philadelphia.....	424
Radiation Pneumonitis: Experimental and Pathologic Observations. Shields Warren, M.D., and Olive Gates, M.D., Boston.....	440
Histologic Changes in the Pituitaries of Parabolic Rats. Isolde T. Zeckwer, M.D., Philadelphia	461

AUGUST 1940. NUMBER 2

	PAGE
Acute Ischemic Necrosis of the Kidney: A Clinicopathologic and Experimental Study. Abraham Penner, M.D., and Alice Ida Bernheim, M.D., New York	465
Histology of Experimental Appendical Obstruction (Rabbit, Ape and Man). Raymond E. Buirge, M.D.; Clarence Dennis, M.D.; Richard L. Varco, M.D., and Owen H. Wangenstein, M.D., Minneapolis.....	481
Tissue Culture as a Diagnostic Aid in the Identification of Atypical Tumors. Machteld E. Sano, M.D., and Lawrence W. Smith, M.D., Philadelphia	504
Relationship of Body Weight to Cancer Incidence. Albert Tannenbaum, M.D., Chicago	509
Squamous Cell Carcinoma of the Eustachian Tube. Harold L. Stewart, M.D., Bethesda, Md., and Marshall M. Lieber, M.D., Philadelphia.....	518
Chemical Analysis of Liver in a Case of Essential Xanthomatosis. Harold Wood, M.D., and Harold Reinstein, Boston.....	533
General Reviews:	
Occurrence and Significance of Congenital Malignant Neoplasms. H. Gideon Wells, M.D., Chicago.....	535
Case Reports:	
Dystrophic Calcification of the Myocardium with Glomerulonephritis. R. M. Bolman, B.M., B.S., Chicago.....	602
Rhabdomyosarcoma of the Corpus Uteri. Charles M. Campbell Jr., M.D., Boston	607
Laboratory Methods and Technical Notes:	
A Rapid Method of Staining Gram-Positive Organisms in Frozen Sections. Aram A. Krajian, Los Angeles.....	614
Notes and News.....	617
Abstracts from Current Literature.....	618
Society Transactions:	
New England Pathological Society.....	627
Buffalo Pathological Society.....	631
Chicago Pathological Society.....	632
New York Pathological Society.....	638
Pathological Society of Philadelphia.....	645
Book Reviews	648
Books Received	650

SEPTEMBER 1940. NUMBER 3

Etiology of Acute Hemorrhagic Pancreatitis, with Special Reference to the Vascular Factors: An Analysis of Autopsies and an Experimental Investigation. C. J. Smyth, M.D., Ann Arbor, Mich.....	651
A Component of Gallstones Insoluble in Ordinary Solvents and Accounting in Part for Their Dark Coloration. H. G. Aronson, M.D., Chicago....	670
Changes in Cartilage and Bone of Immature Female Guinea Pigs Due to Undernourishment, with Consideration of the Processes of Repair Following a Period of Refeeding. Martin Silberberg, M.D., and Ruth Silberberg, M.D., St. Louis.....	675
Wallerian Degeneration in the Sciatic Nerve of the Rat: A Comparative Study with a Silver, the Osmic Acid and the Chlorate-Osmic Acid Methods. Roy Laver Swank, M.D., Ph.D., Boston.....	689
Influence of Sulfanilamide and Sulfapyridine on Experimental Pneumococcal Pneumonia in Rats. David H. Goldstein, M.D., and Irving Graef, M.D., New York	701
Skin Color and Skin Cancer. Joseph Taussig, M.D., and George D. Williams, M.D., St. Louis.....	721
Studies in Experimental Lipoidoses: I. Phosphatides. Armando Ferraro, M.D., and George A. Jervis, M.D., New York.....	731
Case Reports:	
Left Retromesocolic Hernia: Three Additional Cases. Béla Halpert, M.D., New Orleans.....	745
Leiomyosarcoma of the Pleura: A Case with Metastases. Walter A. Stryker, Chicago	750
Teratoma of the Spinal Cord. Mabel G. Masten, M.D., Madison, Wis....	755
Malignant Melanoma of the Palate. H. C. Gotshalk, M.D.; C. F. Tessmer, M.D., and J. W. Smith, M.D., Honolulu, Territory of Hawaii	762

CONTENTS OF VOLUME 30

v

SEPTEMBER—Continued

	PAGE
Laboratory Methods and Technical Notes:	
A Rapid Method of Staining Fat in Frozen Sections with Osmic Acid.	
Aram A. Krajian, Los Angeles.....	766
A Method for Staining Microglia. Juan Negrin Jr., M.D., New York	768
General Reviews:	
Histology of Tumors of the Peripheral Nerves. Nathan Chandler Foot,	
M.D., New York.....	772
Notes and News.....	809
Abstracts from Current Literature.....	810
Society Transactions:	
Chicago Pathological Society.....	824
New York Pathological Society.....	829
Book Reviews	839
Books Received	842

OCTOBER 1940. NUMBER 4

Changes in Number of Circulating Leukocytes in Relation to Spontaneous	
Recovery from Pneumococcc Infection; Experimental Animal Used:	
Guinea Pig. Moyer S. Fleisher, M.D., and G. T. Rich, M.D., St. Louis	843
Atherosclerosis: II. The Lipids of the Serum and Tissues in Experimental	
Atherosclerosis of Rabbits. Sidney Weinhouse, Ph.D., and Edwin F.	
Hirsch, M.D., Chicago.....	856
Phagocytosis of Collagen. George A. Vassos Jr., M.D., New York.....	868
Specificity of Fetal and of Adult Human Hemoglobin Precipitins. Ruth	
Renter Darrow, M.D.; Sophie Nowakovsky, M.D., and Margaret Howard	
Austin, M.D., Chicago.....	873
Microscopic Structure of Striated Muscle in Heat Rigor: The Nodal Multi-	
plication of Striae. Eben J. Carey, M.D., Milwaukee.....	881
Mitosis in Specimens Removed During Day and Night from Carcinoma of	
Large Intestine. William B. Dublin, M.D.; Robert O. Gregg, M.D., and	
Albert C. Broders, M.D., Rochester, Minn.....	893
Chemotaxis of Monocytes Contrasted with That of Polymorphonuclear Leuko-	
cytes and Lymphocytes. Dale Rex Coman, M.D., Philadelphia.....	896
Intravital Staining of Malignant Neoplasms in Man by Evans Blue.	
Alexander Brunschwig, M.D.; Robert L. Schmitz, M.D., and T. Howard	
Clarke, M.D., Chicago.....	902
Case Reports:	
Accessory Pancreas in the Wall of the Gallbladder. Abraham S. Jacobson,	
M.D., New York.....	908
Syphilitic (Gummatous) Pulmonary Arteritis with Rupture Into the Bron-	
chial Tree. A. J. Segal, M.D., Montreal, Canada.....	911
Intrathoracic Pheochromocytoma. Benjamin Philips, M.D., New York	916
Laboratory Methods and Technical Notes:	
Low Temperature Preservation of Gross Specimens. J. Howard Ferguson,	
M.D., Syracuse, N. Y.....	922
Critical Reviews:	
Does Chronic Irritation Cause Primary Carcinoma of the Human Lung?	
Madge Thurlow Macklin, M.D., and Charles C. Macklin, M.D.,	
London, Ontario, Canada.....	924
Notes and News.....	956
Abstracts from Current Literature.....	957
Society Transactions:	
New England Pathological Society.....	972
Buffalo Pathological Society.....	979
New York Pathological Society.....	982
Book Reviews	989
Books Received	992

NOVEMBER 1940. NUMBER 5

Giant Cell Tumor of Bone: Its Pathologic Appearance, Grading, Supposed	
Variants and Treatment. Henry L. Jaffe, M.D.; Louis Lichtenstein,	
M.D., and Robert B. Portis, M.D., New York.....	993
Factors Governing Solubility of Human Gallstones in Dog's Bile. Hans G.	
Aronson, M.D., Chicago.....	1032

NOVEMBER—Continued

	PAGE
Wave Mechanics in Striated Muscle: XVI. Effects of Experimental Variations in Temperature and of Microcapillarity on the Cross Striations in Muscle. Eben J. Carey, M.D., Milwaukee.....	1041
Pigmented Cells of Pia and of Meningeal Tumors. A. E. Taft, M.D., Philadelphia.....	1073
Role of Fixed Tissue Phagocytes in Lipid Metabolism. Edwin F. Hirsch, M.D., and Sidney Weinhouse, Ph.D., Chicago.....	1079
Case Reports:	
Pneumatosis Cystoides Intestini in an Infant. Janvier W. Lindsay, M.D.; E. Clarence Rice, M.D., and Maurice A. Selinger, M.D., Washington, D. C.....	1085
Nodules of Stratified Epithelium in Tubal Mucosa. Alfred Plaut, M.D., and Martin L. Dreyfuss, M.D., New York.....	1089
Laboratory Methods and Technical Notes:	
Plastic Watch Glass Museum Mounting (New Watch Cover and Base Plate). Charles P. Larson, M.D., C.M., Tacoma, Wash., and E. J. Levin, Soap Lake, Wash.....	1093
A Simple Device for Preparing Specimens of Bone Marrow from Small Animals. Edward Kopecky and Herman T. Blumenthal, M.S., Ph.D., St. Louis.....	1095
General Reviews:	
Lysozyme and Its Relation to the Antibacterial Properties of Various Tissues and Secretions. Richard Thompson, M.D., New York.....	1096
Notes and News.....	1135
Abstracts from Current Literature.....	1136
Society Transactions:	
Pathological Society of Philadelphia.....	1150
Buffalo Pathological Society.....	1153
Book Reviews.....	1156
Books Received.....	1158

DECEMBER 1940. NUMBER 6

Changes in the Arteries in the Walls of Tuberculous Pulmonary Cavities. Robert Charr, M.D., and J. Woodrow Savacool, M.D., Philadelphia...	1159
Effect of Exposure to High Oxygen Tension on the Lungs and Heart of the Rat. Donald J. Rehbock, M.D.; Mary Ruth Oldt, M.D., and H. M. Dixon, M.D., Cleveland.....	1172
Effect on the Prostate Gland of Occlusion of Its Ducts. Charles Huggins, M.D., and Philip Johnson Clark, M.D., Chicago.....	1178
Influence of Heptaldehyde on Carcinogenic Action of Methylcholanthrene. Christopher Carruthers, Ph.D., St. Louis.....	1184
Effects of Yellow Phosphorus and Arsenic Trioxide on Growing Bones and Growing Teeth. Carroll O. Adams, M.D., and Bernard G. Sarnat, M.D., Chicago.....	1192
Diplomyelia (Duplication of the Spinal Cord). R. Yorke Herren, M.D., Ph.D., Portland, Ore., and Jesse E. Edwards, M.D., Boston.....	1203
TriPLICATION of the Large Intestine. Alan W. Gray, M.D., C.M., Montreal, Canada.....	1215
Case Reports:	
Spinal Epidural Abscess Associated with Actinomycosis. Newton Krumdieck, M.D., and Lewis Stevenson, M.D., New York.....	1223
Pyelonephritis with Bilateral Strictures of Renal Calices and Associated Hypertension. Delbert E. Siler, M.D., Eloise, Mich.....	1227
Glomerulus-like Neoplastic Structures in a Carcinoma of the Kidney. Milton D. Bosse, M.D., Pittsburgh.....	1235
Laboratory Methods and Technical Notes:	
Use of Solid Carbon Dioxide (Dry Ice) in the Preparation of Museum Specimens. Roland E. Bieren, M.D., Baltimore.....	1240
A Combined Stain for Fat and Elastic Tissue. Claudia French, New York.....	1243
General Reviews:	
Properties of Cancer Cells. E. V. Cowdry, Ph.D., St. Louis.....	1245
Notes and News.....	1275
Abstracts from Current Literature.....	1276
Society Transactions:	
Pathological Society of Philadelphia.....	1293
Book Reviews.....	1295
Books Received.....	1298
General Index.....	1299

SPECIAL NUMBER

DEDICATED TO

DR. S. BURT WOLBACH

Shattuck Professor of Pathology, Harvard Medical School

BY

HIS FORMER AND PRESENT PUPILS AND ASSOCIATES

IN THE DEPARTMENT OF PATHOLOGY

ON THE OCCASION OF HIS

SIXTIETH BIRTHDAY

JULY 3, 1940





S. I. Bunt Volbach





ARCHIVES OF PATHOLOGY

VOLUME 30

JULY 1940

NUMBER 1

COPYRIGHT, 1940, BY THE AMERICAN MEDICAL ASSOCIATION

Foreword

During the historic American trek to the far West the covered wagons crossed the Missouri in a dozen places, but the routes converged to the "great island" in the bed of the Platte, in eastern Nebraska. To be born right there, in Grand Island, near the old Oregon and California trail, and to grow up hearing not only the local tales of the perils and courage and pluck of the pioneers but also the romantic legends of such picturesque figures as Calamity Jane and Wild Bill Hickok, to spend one's early years on the buffalo ranges where cattle and horses and cowboys gradually replaced the ancient herds, to know the excitements of pursuing wild game on the prairies and in the mountains—what conditions more stimulating for enterprise and adventure could a boy have than that! And they had their effects. Hunting and horseback riding became Burt Wolbach's chief recreations; exploring Indian relics and listening to Indian lore were his diversions; trying experiments at home and confronting his teachers in school with novel solutions of problems in algebra and geometry were early signs of his independence in thought and action. It is reported that when he was about to take his entrance examinations for Harvard he had had no instruction in two of the required subjects; a brief period of intensive, self-reliant study enabled him to pass them both.

A boyhood and youth spent unconventionally in this fresh, "abundant" West; associations with the alert and hardy men whose vigorous enterprise and resourcefulness helped to build its civilization; interests which called forth careful studies of living things, their habits and their surroundings; devotion to tools of precision which can be used effectively only if used with understanding—such were the experiences which formed a sound basis for Dr. Wolbach's scientific career.

The geographic frontiers of our country have slowly and inevitably vanished. The pioneers can no longer push onward into unexplored territory. There are, however, other and perpetual frontiers, the twilight zones between our world of enlightenment and the vast dark realm of our ignorance. And excursions into that dark realm call for the same rare combination of qualities which characterized the early venturers into the American West—initiative, ingenuity, resourcefulness and a

calm and tenacious confidence in the possibility of achievement. There may be no demonstrable relation between youthful experiences in which these qualities were eminently exemplified and esteemed and adult habits of action, but when there is consistency in the sequence it suggests a persistent influence. Certainly Dr. Wolbach's upbringing on the western plains was a good preparation for his later extensive excursions as an outdoor pathologist. When a youngster he was a free-roving naturalist, unconsciously making ready, in years ahead, to wander widely in the world—in Africa, Mexico, Montana and Poland—to learn about diseases in their normal surroundings. Thus disciplined by circumstances to observe life in the open, to meet and adjust himself to all sorts of places and men, he became an effective field student of his science.

By good fortune Dr. Wolbach approached pathology by way of a pertinent accessory discipline. While still a medical student he completed his first research, conducted under Harold C. Ernst in the department of bacteriology of Harvard Medical School. Formal training in pathology he obtained from William T. Councilman, whom he was to succeed, and from Frank B. Mallory at the Boston City Hospital (1903-1908). In that well known center of investigative concern with pathologic anatomy and histology he had a rare opportunity to broaden his experience and to acquire proficiency in staining technic and in histologic interpretation. Corresponding with these shifts of interest between bacteriology and pathology were his academic appointments. First he was assistant (1905-1906) and instructor (1906-1908) in pathology at the Harvard Medical School. Then, in 1908, at the Albany Medical College he was made adjunct professor of pathology and bacteriology. There followed two delightful and valuable years spent as pathologist to the Montreal General Hospital, an association which bound him with strong ties to both the city and the hospital which he served. His success as the head of an important hospital laboratory and his persistent activities in research led Dr. Ernst to urge the recall of his former student to Harvard. Dr. Wolbach, not yet 31 years old, was first appointed assistant professor (1910-1914), later (1914-1916) associate professor, of bacteriology, and thereafter associate professor of pathology and bacteriology. Thus, for nearly twenty years he was engaged in activities requiring thoughtful consideration of the living pathogenic agent and the functional and structural disturbances induced by it. Only in 1922, when he received the Shattuck professorship of pathologic anatomy, did the formal oscillation between duties to both pathology and bacteriology finally cease. Extensive and intimate experience with these closely related fields gave Dr. Wolbach a binocular insight into the nature of transmitted diseases, highly useful to him as a teacher and as an investigator. A specific lesion signified a definite

etiologic factor, even though the factor was not yet demonstrated. It was that firmly based conviction which constantly supported him throughout his arduous and baffling search for the parasite of Rocky Mountain spotted fever—a search which resulted not only in the discovery of the parasite but also in a definite characterization of the pathology of the disease.

Dr. Wolbach's interest in photography and his knowledge of the physics and chemistry of photographic processes have been reflected in remarkable photomicrographs, which have illustrated his publications. During his years of service at the Boston City Hospital he experimented with photographic devices and invented new methods in the technic of objective recording of microscopic fields. The delicacy of shading and the clarity of his pictures have brought them a wide reputation. Not infrequently they display a choice of balanced features and an appreciation of design which give them artistic as well as scientific value.

An investigator is commonly judged by the significance of his investigations. Although Dr. Wolbach's earliest papers included a considerable number of case reports, he soon revealed a gift for selecting important general subjects for attention. For example, a pioneer research on chronic roentgen ray dermatitis and the initial stages of roentgen ray carcinoma (1909) is still recognized for its excellence, since almost nothing has been added to the subject during the past thirty-one years. His cooperation with Dr. John L. Todd in a scientific expedition to the Gambia (1911) led to an acquaintance with tropical disease in its natural circumstances and to reports on protozoan parasites which were involved. These and other rather diverse studies were, however, preliminary to his later more consistent researches. Rarely does one man make original and fundamental contributions to more than one field of knowledge; it is Dr. Wolbach's distinction to have to his credit contributions, marked by those high qualities, in two ranges of interest, with which his name has become especially associated—the etiology and pathology of rickettsial diseases and the pathology of states of vitamin deficiency.

The report of the critical and rigorously controlled observations and experiments carried on by Wolbach and his collaborators, especially John L. Todd and F. W. Palfrey, in Poland (1920) has become a classic in the study of disease. These observations and experiments led definitely to the conclusion "that the virus of typhus is not separable in the louse from *Rickettsia prowazeki*." The importance of this testimony is understood when it is compared with the inferences of competent pathologists and parasitologists who, in spite of accumulated circumstantial evidence, had opposed, within two years (1918), the conclusion that any rickettsias were pathogenic. Undoubtedly an

admirable preparation for strictly conducted tests on the etiology of typhus was Dr. Wolbach's painstaking analysis of the role of *Derma-centroxenus rickettsi* and the tick in the transmission of Rocky Mountain spotted fever. Only by carrying to Poland "clean" lice in small boxes on their own bodies did Wolbach and Todd provide the rigid conditions for conclusive demonstration. When such lice acquired the typical parasite of typhus, and only that, if permitted to bite patients with the disease, and when in turn inoculation of the viscera of these lice, thus infected, conveyed the disease to guinea pigs, whereas inoculation of the viscera of seven lice from infected boxes that failed to convey the diseases were found free of the parasite, the relation of the virus of typhus to *Rickettsia prowazeki* was definitely proved. Dr. Wolbach's thorough and convincing observations and experiments on Rocky Mountain spotted fever and on typhus laid a firm foundation for later researches on the rickettsia type of micro-organisms; indeed, the later researches in this field may properly be regarded as justified by the proofs which he had established.

The contributions to vitamin research made by Dr. Wolbach and his associates, notably P. R. Howe, disclosed previously unappreciated opportunities for cooperation between biochemists, physiologists and pathologists. Prior investigations had laid emphasis especially on the functional disturbances produced by vitamin deficiency; and although there had been scattered reports of pathologic effects produced by lack of essential food elements, the common features of the disturbances had not been detected, nor had the details of degeneration and repair been carefully followed. Extensive studies by Wolbach and Howe allowed certain generalizations to be laid down. First, lack of vitamin A disturbs selectively epithelial tissues, inducing primary atrophy, followed by reparative proliferation of basal cells which become stratified and keratinized; other effects are consequences of the epithelial changes. Second, lack of vitamin C results in a failure of cells to produce and maintain intercellular substances, e. g., collagen and the matrices of bone, dentine and cartilage. The profound and specific alterations in structure that can be induced by vitamin deficiencies, and the astonishingly rapid reversal or renewal of processes, directed toward the normal status of the morbid tissues, that occurs when a proper diet is provided, excited Dr. Wolbach's imagination. In his highly stimulating DeLamar Lecture of 1937 he pointed out the wide-ranging value of vitamin research as a means of "manipulating" morphologic sequences.

The investigative achievements of Dr. Wolbach are doubly to his credit because of the heavy demands on his time imposed by numerous routine and special hospital duties. For the past quarter-century he has been pathologist of the Children's Hospital, Boston; for nearly twenty years he has been pathologist of the Peter Bent Brigham Hos-

pital; in addition he has provided the pathologic services at the Boston Lying-in Hospital and the Free Hospital for Women. To be sure, these assumed obligations, though time consuming, have been valuable both to him and to medicine. The Children's Hospital, for example, exposed to his quick insight the little known pathology of early life. Nutritional disorders in the young offered immediate illustrations of the phenomena of avitaminosis which he had observed experimentally and which he could explain and treat with clear understanding. His interest in the early appearance of neoplasia has been responsible for a reduction of the mortality caused by malignant tumors in childhood. Cooperative studies with physicians and surgeons resulted from these specific clinical interests. At the regularly recurring pathologic conferences in the hospitals with which he has been connected Dr. Wolbach has had occasion to manifest, quite unconsciously, characteristics which have made him an outstanding investigator and teacher. An immense personal knowledge of both bacteriology and pathology, combined with a modest readiness to confess ignorance when the issue is obscure, a meticulous accuracy of observation and cautious and limited inferences therefrom, luminous and stimulating discussion of puzzling problems presented by a case—such are the impressions he has left on hundreds of young interns who have had the privilege of coming under his inspiring influence. A smaller number of advanced students have received from him the special training of the pathologist. Here, as a leader in research, his remarkable skill and ingenuity have been displayed, here his insistence on highest standards of perfection has been emphasized, here within the bounds of possible proof he has let his imagination have free play, and here he has given insight and encouragement to promising future contributors to medical science. In such contacts no bounds can be set to the value of a stimulating teacher.

Equally fortunate have been Dr. Wolbach's gifts as an instructor of medical undergraduates. Approaching the course in pathology as a fellow student, avoiding always the dogmatic attitude of authority, he gives them something more than a mere catalogue of facts. He presents a critical evaluation of theories and observations important for revealing a useful point of view and as a preparation for the clinical years. Ever stressed in his teaching is the correlation of pathology with the accessory disciplines of physiology and chemistry. His carefulness and patience during informal discussions in the laboratory leave lasting impressions on his student listeners.

In personal qualities Dr. Wolbach seems, at first meeting, notable for reserve and quiet dignity and the appearance of emotional poise. He is deliberate and unhurried in speech and action. These obvious features might obscure to the casual acquaintance the underlying warmth of his nature. The cordial friendliness and hospitality of his home life

have brought memorable pleasures to a host of his students and colleagues. And wherever he has labored—in the various hospitals and laboratories of Boston, in Albany and in Montreal—his kindness and his generous helpfulness have made for him devoted friends. When confronted with a problem, whether in an autopsy room or at a faculty meeting, his judgment is thoughtful and well reasoned; once convinced that a decision is warranted and just, he advocates it with complete fearlessness. There is no sham or false front in any aspect of his character. Loyalty is, perhaps, his crowning trait. It is manifest not only in staunch devotion to those whom he has long known but also in a tenacious hold on old fidelities. He likes the feel of a well tested gun (he is a crack shot!) and the nice balance of a good ax, instruments which he has often used and has found serviceable. A bookplate of which he dreamed recalls affectionate memories of his Nebraska boyhood—a horned lark perched on a dry buffalo skull, with the wide prairies stretching away in the background. And yet his heart does not belong wholly to the Nebraska of his earliest associations. Like many another westerner transplanted to New England, he has deep appreciation of the beauty of the region and admiration for its strong traditions of public service and respect for individual opinion and intellectual independence. He showed his conversion to the East by marrying a native of Massachusetts and establishing a home overlooking the Sudbury valley, not far from Boston. There he and his family enjoy many of the pleasures of country living. To see Burt Wolbach and his favorite horse, each nuzzling the other, is a beautiful revelation of both human and equine nature. From the time of his youth the horse has been a close companion. A morning ride, rain or shine, still keeps him physically fit for the strenuous work of the day—teaching, attending to routine duties in school and hospitals, engaging eagerly in various research projects, and fulfilling his deep sense of obligation to the future by guiding his disciples along old paths and toward fresh ventures in pathology. In all these diverse activities his persistent vigor foretells that the sciences in the service of which he has spent his years will continue to benefit from his attentive zeal.

WALTER B. CANNON.

RHEUMATIC DISEASE OF THE TRICUSPID VALVE

MARK D. ALTSCHULE, M.D.

AND

EDWARD BUDNITZ, M.D.

BOSTON

The relative rarity and the striking clinical and pathologic manifestations of organic disease of the tricuspid valve have stimulated interest so that a number of very thorough clinical discussions of the disease are available in the literature.¹ Observations on the pathologic physiology of this syndrome are, however, few; several years ago studies of the cardiovascular dynamics in a single case were described in some detail.² The purpose of the present report is to describe additional physiologic studies in this and another case, to record the postmortem observations in both and to correlate all the observations with the clinical manifestations.

REPORT OF CASES

CASE 1.—M. R., a 32 year old white American housewife, first entered the Beth Israel Hospital Jan. 29, 1936, complaining that for ten years she had experienced dyspnea on exertion. The past and the familial history were irrelevant. Eleven years before admission the patient had migratory polyarthritis over a period of four months, associated with pain and fever, but no swelling or redness of the joints, forcing her to remain in bed. Following this illness, she was advised to curtail her activities because she had a cardiac murmur, but she disregarded this advice. About a year later she began to notice easy fatigue, slight dyspnea and slight palpitation on exertion. She also noted slight swelling of the ankles, particularly of the left, at the end of the day. Her condition remained stationary until six years before admission, when, because of increase of symptoms, she entered another hospital, where she was digitalized and discharged improved after a stay of eleven days. About seven months before the present admission she began to experience a dragging sensation and noted the appearance of a "big lump" in the epigastrium. Simultaneously she began to experience increasing breathlessness and frequent eructations of gas, with, however, no nausea or vomiting. During the four months preceding admission she had five or six attacks of severe cramping pain in the

From the Medical Service and Medical Research Laboratories, Beth Israel Hospital, Boston, the Cardiac Service, Worcester City Hospital, Worcester, and the Department of Medicine, Harvard Medical School, Boston.

1. (a) Herrick, J. B.: *Boston M. & S. J.* **136**:245, 1897. (b) Herrick, W. W.: *Arch. Int. Med.* **2**:291, 1908. (c) Dressler, W., and Fischer, R.: *Klin. Wchnschr.* **8**:1269 and 1316, 1929; *Ztschr. f. Kreisslaufforsch.* **22**:188, 1930. (d) Fingerhuth, M., and Bickenbach, O.: *Deutsches Arch. f. klin. Med.* **175**:577, 1933. (e) Zeisler, E. B.: *Am. Heart J.* **8**:697, 1933. (f) Friedlander, R. D., and Kerr, W. J.: *ibid.* **11**:357, 1936; **15**:625, 1938.

2. Altschule, M. D., and Blumgart, H. L.: *Am. Heart J.* **13**:589, 1937.

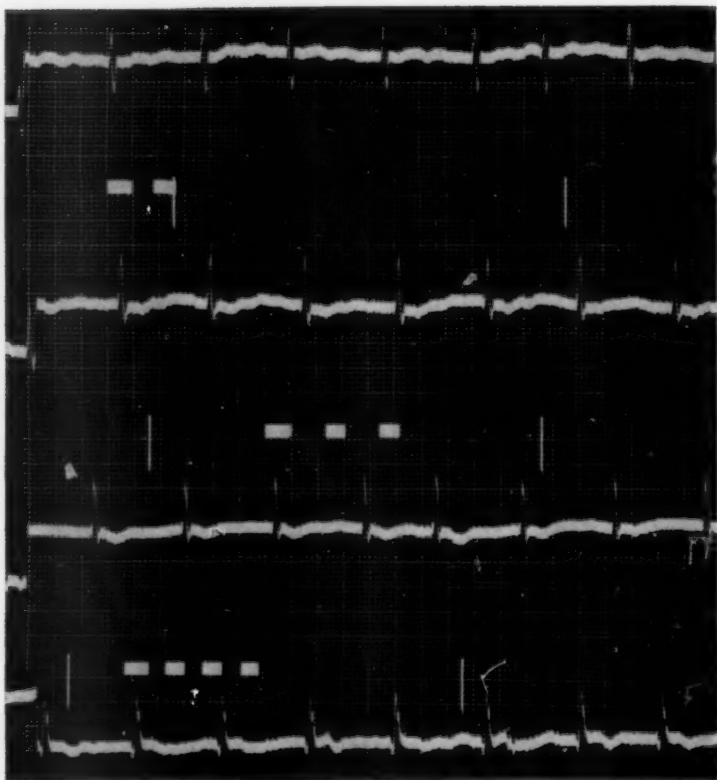


Fig. 1.—Electrocardiogram in case 1, showing right axis deviation and auricular fibrillation.

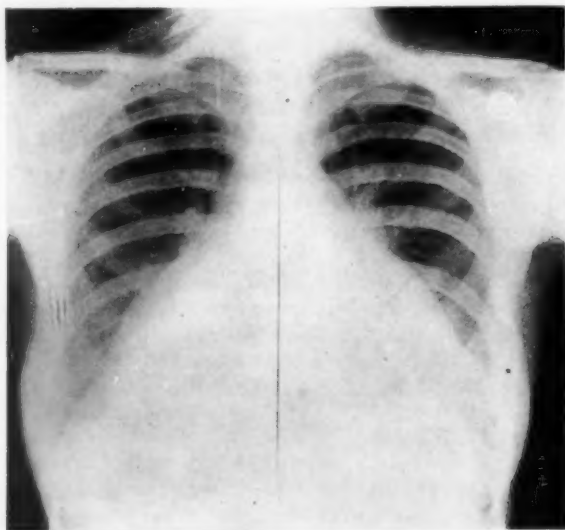


Fig. 2.—Roentgenogram of heart in case 1.

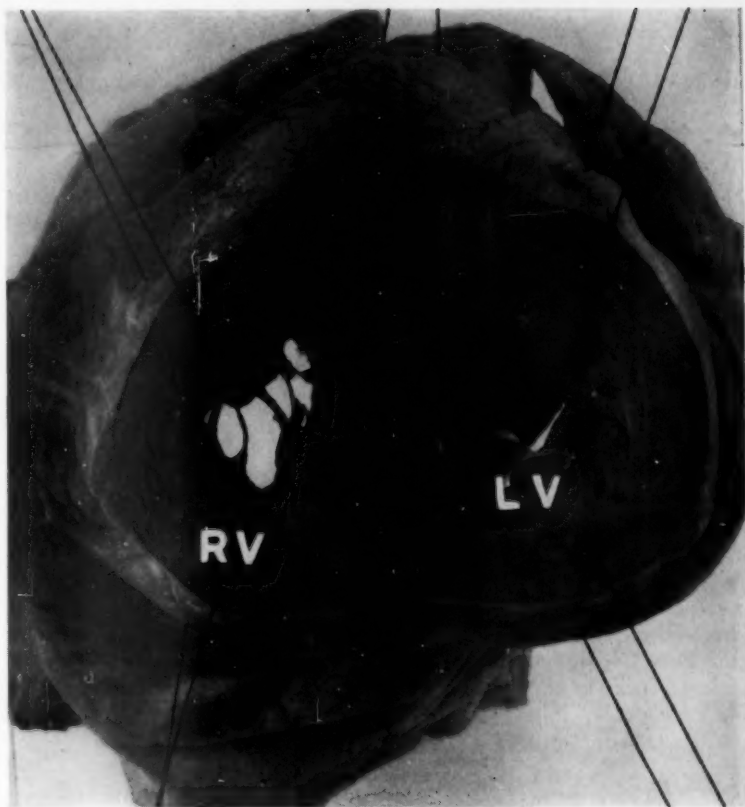
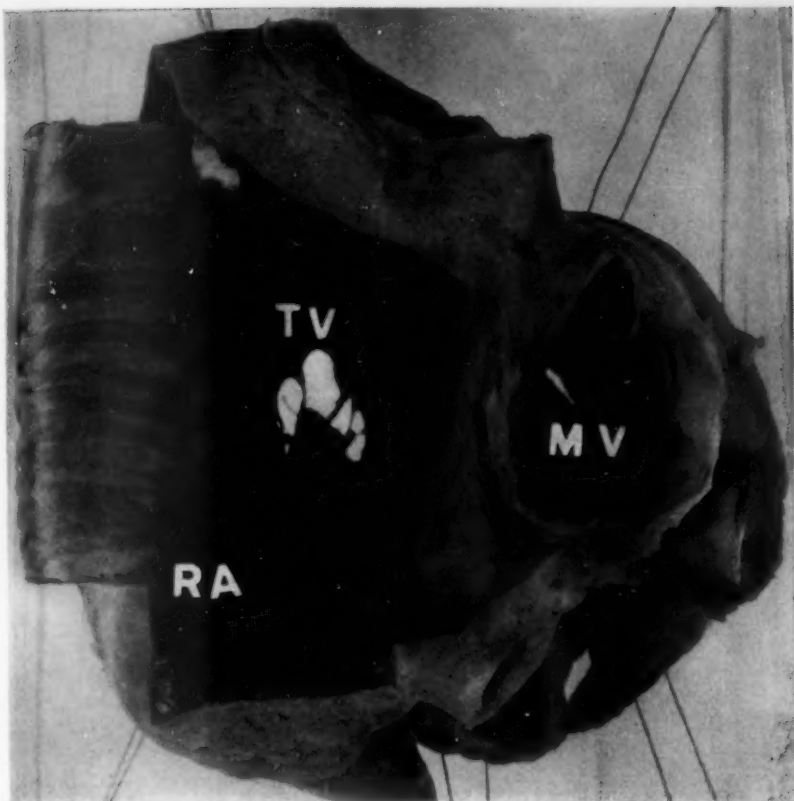


Fig. 3.—Upper: Heart in case 1 from above. *TV* is the thickened fibrosed tricuspid valve; *MV*, the mitral valve; *RA*, the dilated right auricle with thinned wall. Lower: Heart from below. *RV* is the right ventricle; *LV*, is the left ventricle. Both ventricles are hypertrophied.

right upper quadrant of the abdomen which radiated to the left upper quadrant and required morphine for relief. During this period orthopnea appeared, and edema became more persistent, though at no time was it more than moderate. The patient had been up and about but was compelled to remain in bed for a period of three weeks, after which she led a bed and chair existence. A month before admission she noted the onset of continuous pain in the right shoulder. At no time did she have cough, fever, pain in the chest or paroxysmal dyspnea.

Physical examination revealed a fairly well developed and well nourished young woman, with a slight icteric tint to the skin and slight cyanosis of the lips. The veins of the neck, face, forehead, arms and hands were dilated and pulsated synchronously with the heart. The veins over the face and forehead were from 0.5 to 1.0 cm. in diameter. Ophthalmoscopic examination gave negative results except for dilatation and pulsation of the retinal veins. The heart was markedly enlarged to both right and left, the borders of dulness being, respectively, 11 and 12 cm. from the midsternal line. Over the area of the mitral valve a diastolic thrill and a faint systolic thrill were felt. A systolic thrill was also palpable over the area of the aortic valve. A rough systolic murmur and a rolling diastolic murmur were audible at the apex. A loud, rough systolic murmur and a loud whistling decrescendo diastolic murmur were heard over the lower end of the sternum and were transmitted toward the right. A rough systolic murmur and a short early diastolic murmur were also audible over the third left interspace. The second sound over the area of the aortic valve was absent. The rhythm was completely irregular. The heart rate was 70, with no pulse deficit. There was some dulness over the base of the right lung posteriorly. No rales were heard. The liver was enlarged to the umbilicus and was tender and pulsating. The pulsations were systolic in time only. The spleen was not palpable. No capillary pulsation or Corrigan pulse was present. There was no edema of the ankles, legs, sacrum or back. The arterial blood pressure was approximately 140 mm. of mercury systolic and 84 mm. diastolic.

The clinical diagnoses were: rheumatic heart disease; stenosis and insufficiency of the tricuspid, mitral and aortic valves, and auricular fibrillation.

Electrocardiographic tracings showed auricular fibrillation, right axis deviation, diphasic T_2 and inverted T_3 and T_4 (fig. 1).

Four examinations of the urine revealed a specific gravity ranging between 1.020 and 1.030. Urine taken without catheterization was normal except for the constant presence of small amounts of albumin. The red blood cell count was 4,350,000 per cubic millimeter; the hemoglobin was 80 per cent (Sahli). The white blood cell count on three occasions was between 6,100 and 8,000 per cubic millimeter. The differential count and smear were normal. The stool revealed no abnormality. The blood nonprotein nitrogen was 29 mg. per hundred cubic centimeters; the blood sugar, 92 mg.; the serum total protein, 7.4 Gm. and the serum cholesterol, 189 mg. The Hinton and Kahn reactions of the blood were negative. On admission the icteric index was 20, and the result of the van den Bergh test was 2.3 mg. per hundred cubic centimeters. These fell, respectively, to 12 and 1.3 during the next two weeks. The galactose tolerance test was negative.

The patient was given a house diet, with fluids to 1,500 cc. and 0.1 Gm. of digitalis daily. She was discharged improved after a stay of seventeen days. Eleven months later she was readmitted because of rapid development of edema and abdominal distention and aggravation of dyspnea and orthopnea during the preceding three weeks.

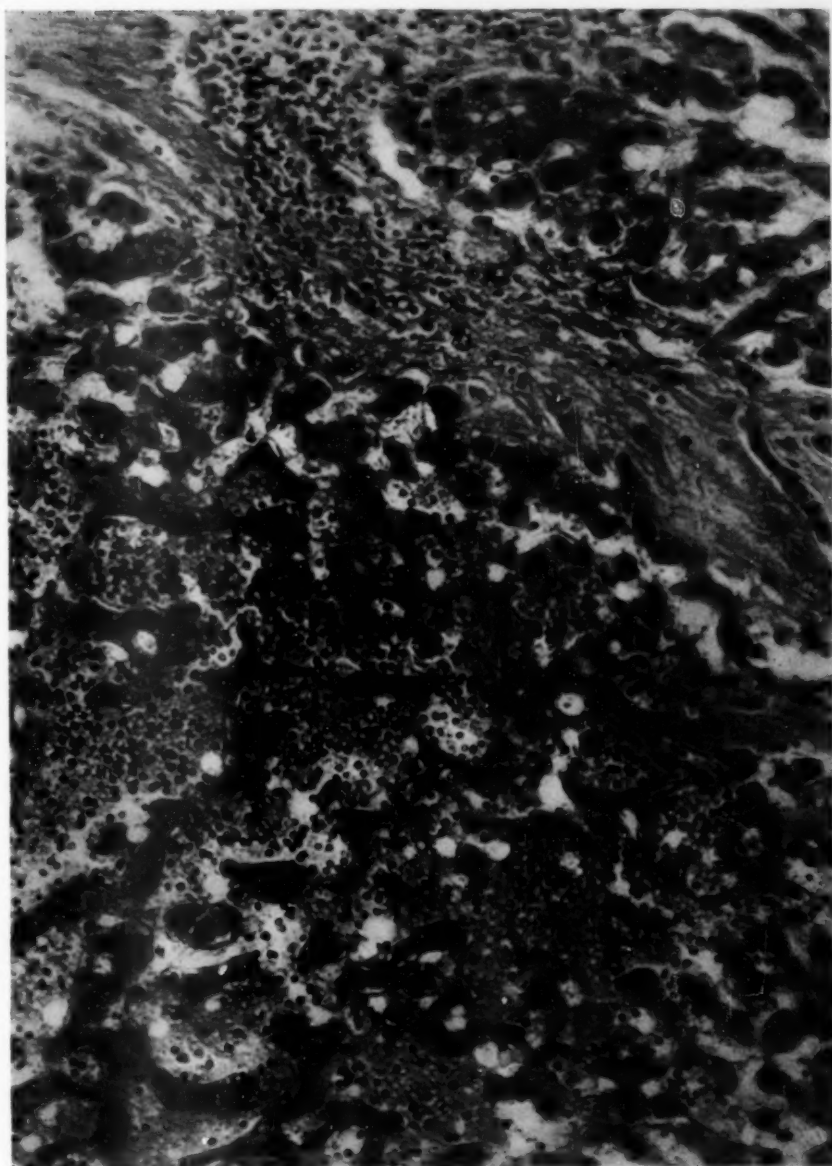


Fig. 4.—Liver in case 1. Note the marked congestion and moderate portal fibrosis; $\times 215$.

The physical findings on readmission were as previously noted except that the veins of the face and neck were more markedly distended, cyanosis was increased, rales were heard over the bases of the lungs, there were signs of ascites, and marked edema was present over the legs and sacrum.

Electrocardiographic tracings showed auricular fibrillation and right axis deviation with inversion of T_2 , T_3 and T_4 .

The urine in eighty-four examinations showed a specific gravity ranging from 1.006 to 1.030, with most of the readings above 1.020. Almost all specimens contained small amounts of albumin and occasional leukocytes; a few specimens also contained bile. Erythrocytes were noted occasionally. Twenty red blood cell counts ranged between 3,000,000 and 3,700,000 with hemoglobin values of 70 to 80 per cent (Sahli). The reticulocyte count was between 4 and 5 per cent on several examinations. The leukocyte count ranged between 6,000 and 13,000, with most values below 9,000. The blood nonprotein nitrogen concentration was 46 mg. per hundred cubic centimeters on admission and fell promptly to approximately 30, a level about which it fluctuated thereafter. The icterus index ranged between 15 and 30 throughout, with serum bilirubin values of 1.4 to 2.9 mg. per hundred cubic centimeters. The serum protein was 8.3 Gm. per hundred cubic centimeters on admission, falling terminally to 5.2 Gm.

The patient was in the hospital for twelve months, during which time the signs of congestive heart failure failed to respond to treatment and slowly grew worse. Various diuretics were found only partially effective, and abdominal paracentesis was done every month or two. The physical findings never changed markedly from those on admission except that the edema slowly spread to involve the entire trunk and purpura appeared over the lower parts of the legs, persisting during the last six months of the patient's life. The patient died suddenly while in general anasarca.

Postmortem Examination.—This examination was performed fifteen hours after death. The pericardial contents occupied most of the thorax, the lungs being markedly compressed. The greatest transverse diameter of the chest was 24 cm.; 22 cm. was occupied by the unopened pericardium. The latter measured 18 cm. longitudinally. The pericardium contained 1,300 cc. of slightly turbid yellow fluid. The heart unopened appeared enlarged bilaterally; however, the right auricle was larger than the entire rest of the heart. The organ weighed 430 Gm., and both ventricles appeared slightly hypertrophied, measuring 1.5 and 0.8 cm. in thickness, respectively. The right auricular wall, however, was paper thin. The right auricle had a capacity of 800 cc.; the left auricle, 100 cc.; the right ventricle, 35 cc., and the left ventricle, 10 cc. The tricuspid valve was thickened and scarred, and showed complete fusion of the cusps. Its orifice barely admitted two fingers and measured 2.5 by 1.5 cm., which is considerably less than normal. The mitral valve was similarly affected and showed, in addition, marked shortening of the chordae tendineae. Its orifice was a narrow slit 2 cm. long. There were also fusion and thickening of the aortic cusps, with some degree of stenosis and incompetence. The pulmonary valve was normal. On microscopic examination the ventricular myocardium was found to contain scattered patches of fibrosis, many of them fusiform in shape with small arteries in the center; these contained a few symmetrically arranged chronic inflammatory cells and suggested healed Aschoff nodules.

The liver weighed 1,240 Gm. and showed irregular fibrous thickening of the capsule. There was some resistance to cutting. Sections through it revealed red central areas in the midst of yellowish green lobules and irregular narrow bands of fibrous tissue throughout. On microscopic examination there was an extreme

degree of central congestion, resembling in many areas hemangioma. The fibrosis noted grossly was found to be mainly portal.

There was marked distention with increase in diameter of all the veins.

The other gross findings consisted in general edema, cyanosis, marked congestion of all the viscera, 3,500 cc. of fluid in the abdomen, 700 cc. in the right pleural cavity and 300 cc. in the left. Microscopic examination confirmed the gross findings and revealed, in addition, many hemosiderin-laden phagocytes in the lungs.

CASE 2.—G. H., a 42 year old white American machinist, entered the Beth Israel Hospital Jan. 19, 1939, complaining of dyspnea and swelling of the abdomen. He had chorea at the age of 9, and during a routine physical examination at the age of 16 was told that he had a cardiac murmur. However, he had no symptoms until twelve years later, fourteen years before admission, when he had an attack of sudden weakness and coughed up some blood-streaked sputum. At this time he first noted pulsations in the veins of his neck. He entered a hospital and was digitalized, with marked improvement, and was able to return to his previous level of activity with only mild dyspnea. Six years before his admission an inguinal

TABLE 1.—Cardiac Measurements from Roentgenograms in Two Cases of Rheumatic Disease of the Tricuspid Valve (Taken at a Distance of Seven Feet [213 Cm.]. Peripheral Signs of Congestive Failure Not Present)

	Case 1, Cm.	Case 2, Cm.
Midsternum to right.....	10.0	6.1
Midsternum to left.....	11.3	14.7
Total transverse diameter.....	21.3	20.8
Diameter of great vessels.....	8.5	7.0
Length	19.2	21.0
Base	17.0	14.2
Transverse diameter of chest.....	24.6	32.0

hernia developed, which attained a large size in a period of a few weeks. During the operation for its repair a good deal of fluid ran out through the incision. He was never able to go back to work after this, as he was troubled by anorexia, dyspnea and the coughing up of blood-streaked sputum. Five years before admission his abdomen and legs swelled, and the veins of his neck became markedly engorged. Hospitalization resulted in marked improvement, which persisted for about six months, after which time it became necessary to do abdominal paracentesis every two months. In the five years preceding his admission to the Beth Israel Hospital his dyspnea and weakness slowly became worse.

Physical examination on admission revealed a well developed and moderately well nourished cyanotic, slightly icteric middle-aged man with marked engorgement of the veins of the head and neck; the latter were pulsating strongly, particularly on the right, where a thrill was also palpable. In the region of the right internal jugular vein immediately above the scapula there was a bulge which in diastole measured 3 cm. in diameter. This bulge pulsated. Ophthalmoscopic examination disclosed nothing except pulsation of the retinal veins. The venous pulsations were systolic. The heart was markedly enlarged, the limits of cardiac dullness being 17 cm. to the left and 8 cm. to the right of the midsternal line. The rhythm was grossly irregular. Over the apex there were systolic and diastolic murmurs, the latter being low pitched and rumbling and associated with a thrill; to the right of the xiphoid process a low-pitched whistling systolic murmur was heard. A soft systolic murmur was heard also over the base of the heart. There was dullness over

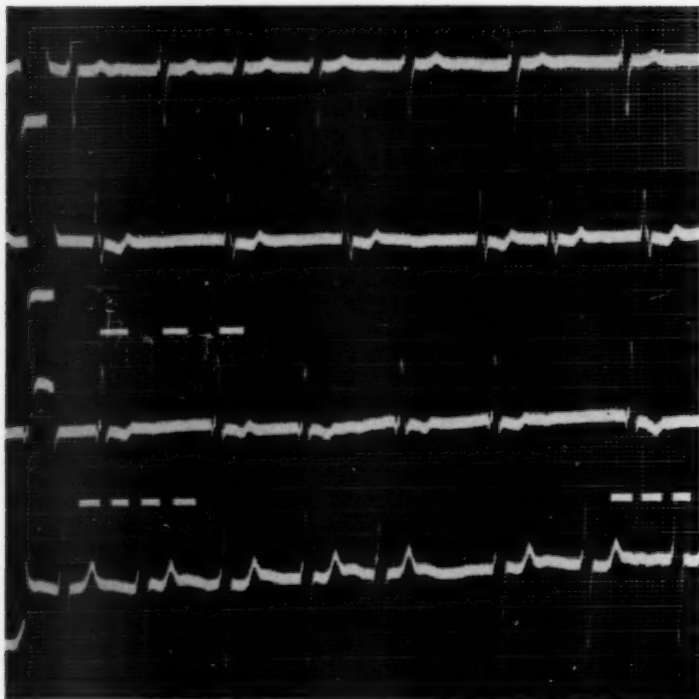


Fig. 5.—Electrocardiogram in case 2, showing right axis deviation and auricular fibrillation.

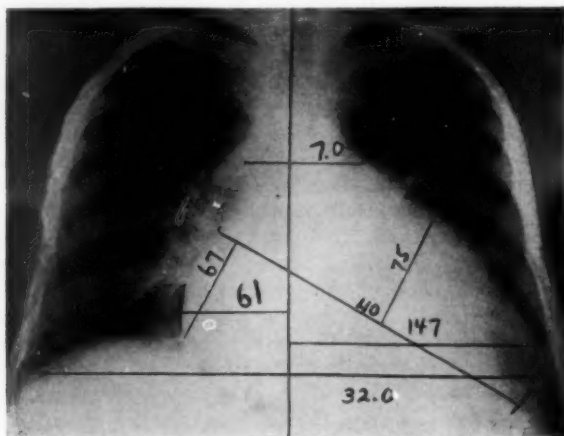


Fig. 6.—Roentgenogram of heart in case 2.

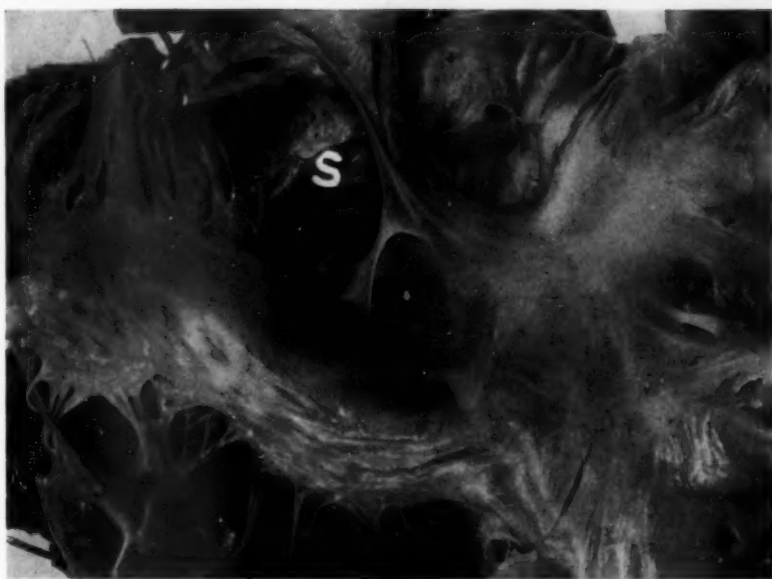
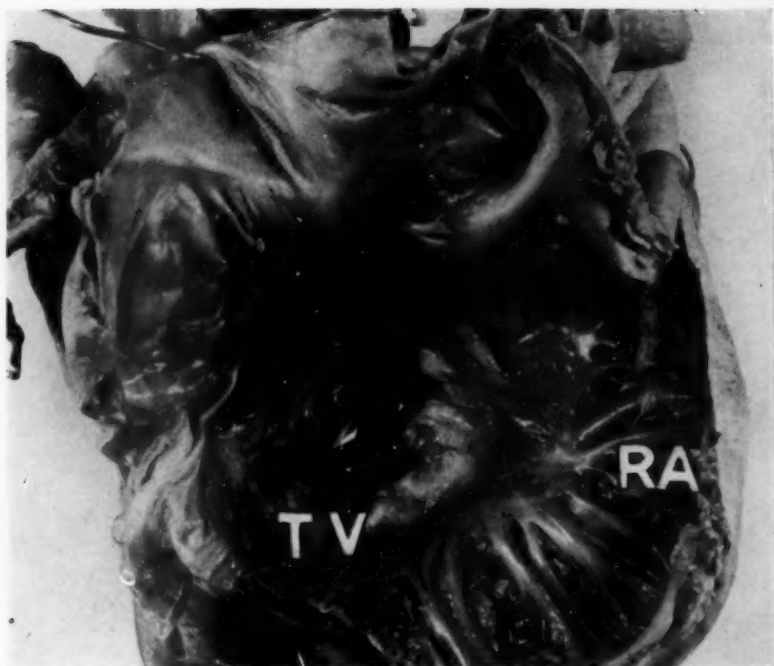


Fig. 7.—Upper: Heart in case 2 from above. *TV* is the thickened, fibrosed tricuspid valve; *RA*, the dilated right auricle with hypertrophied wall. Lower: Greatly dilated coronary sinus (*S*) in same heart.

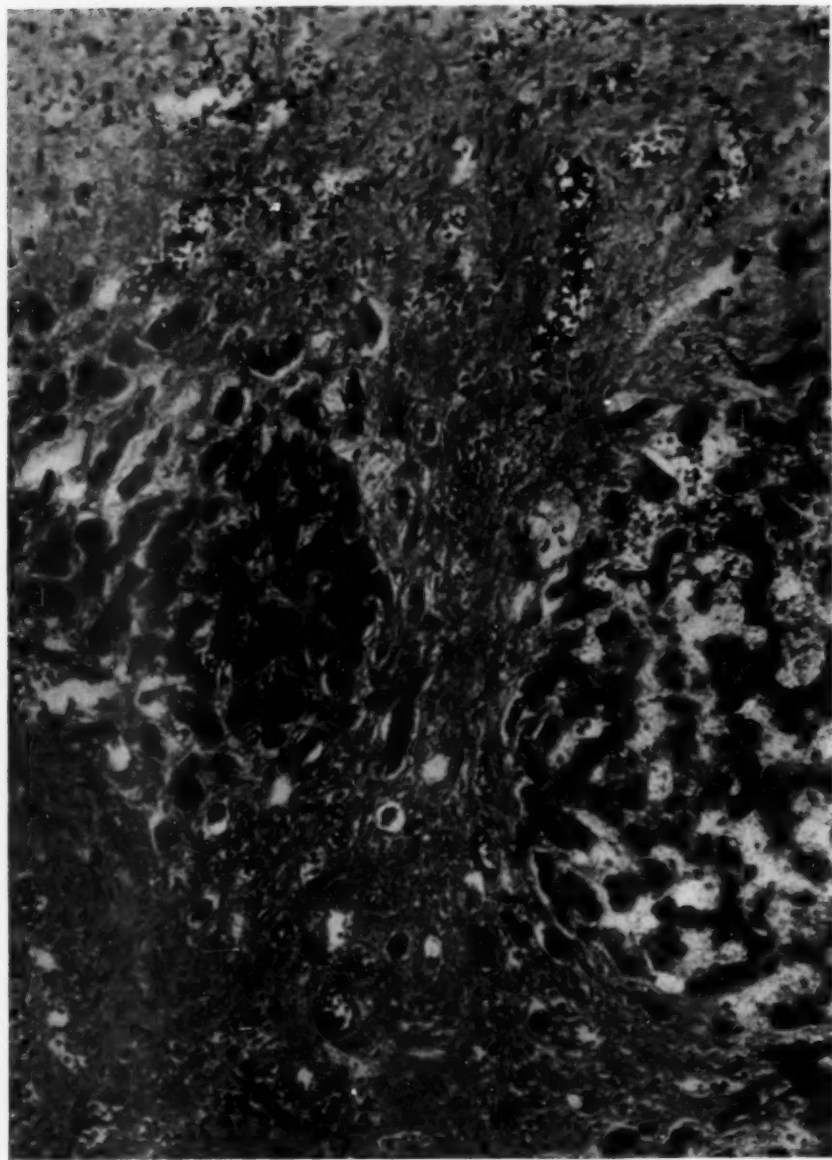


Fig. 8.—Liver in case 2. Note the moderate congestion and marked fibrosis;
×215.

the base of the right lung, with a few rales audible over the bases of both lungs. The liver was enlarged 7 cm. below the costal margin and pulsated synchronously with the heart. The spleen was felt 5 cm. below the left costal margin. A shifting dullness and a fluid wave could be elicited on examination of the abdomen. There was slight edema of the lower parts of the legs. The blood pressure was approximately 110 mm. of mercury systolic and 70 mm. diastolic.

The clinical diagnoses were: rheumatic heart disease, insufficiency of the tricuspid valve, stenosis and insufficiency of the mitral valve and auricular fibrillation.

The urine, examined nine times, revealed a specific gravity ranging between 1.006 and 1.020. All specimens contained small amounts of albumin and occasional leukocytes and erythrocytes. The red blood cell count was between 4,200,000 and 5,000,000 on three occasions, with hemoglobin content approximately 65 per cent. The white blood cell count ranged between 5,000 and 10,000, with a normal differential count and smear. The reticulocyte count was 0.6 per cent. The serum icteric index was 13. The serum total protein ranged between 5.9 and 6.8 Gm. per hundred cubic centimeters. The blood nonprotein nitrogen level was 39 mg. per hundred cubic centimeters on admission and thereafter was between 55 and 71 mg.

Two electrocardiograms showed auricular fibrillation and right axis deviation. In one of them T_2 and T_3 were diphasic; in the other T_1 and T_4 were inverted.

The patient was given a house diet, with fluids limited to 1,500 cc. daily, and was digitalized. Various diuretics were found to be ineffective until urea was given in daily doses of 70 Gm. This resulted in a decrease in weight from 165 to 143 pounds (74.5 to 65 Kg.) in a period of twenty-three days. The slight rise in blood nonprotein nitrogen which accompanied this medication was without consequent symptoms. The patient was discharged free of ascites and signs of congestive heart failure after thirty-seven days in the hospital. Improvement was maintained for approximately two months, after which time ascites recurred. This was controlled for a time by means of abdominal paracentesis, performed at intervals of two to three months. Edema of the legs appeared and slowly became worse in spite of treatment and complete rest in bed. The patient entered the Worcester City Hospital on Dec. 10, 1939.

The physical findings on readmission were as previously noted except for very marked edema from the waist down and signs of a very large amount of ascites.

The laboratory data were essentially as on the previous admission to the hospital except that the serum protein was 5.4 Gm. per hundred cubic centimeters.

Abdominal paracentesis was performed shortly after admission, with the removal of 10 liters of slightly turbid yellowish fluid. All attempts at therapy were without benefit, and the patient died thirty-three days after admission.

Postmortem Examination.—The autopsy was performed eight hours after death. The pericardium appeared greatly distended, compressing the lungs; this increase in size was due to cardiac enlargement, as only 40 cc. of pericardial fluid was present. The heart was markedly enlarged, weighing 840 Gm. The right ventricle was dilated and markedly hypertrophied, its wall measuring 1.3 cm. in thickness. The left ventricle measured 1.8 cm. in thickness. The dilatation of the right ventricle was associated with an increase in the circumference of the tricuspid ring to 16 cm. The tricuspid valve itself was thickened and fibrosed, and the leaflets were partially fused. The chordae tendineae were somewhat shortened. The valve orifice admitted two fingers only and measured 3 by 2 cm., which is considerably less than normal. There was therefore a considerable degree of stenosis, also

marked incompetence, the latter due both to the organic lesion and to the stretching of the valve ring. The mitral and aortic valves showed marked fibrosis, thickening and fusion of the leaflets, with deposition of calcium. The pulmonic valve was normal. The right auricular wall was thickened to a moderate degree. The right auricle contained 400 cc. and the left 200 cc. The coronary sinus was greatly dilated, its orifice admitting the forefinger freely. Microscopic examination of the myocardium revealed no striking abnormalities.

The venae cavae were markedly dilated, as were the internal jugular veins. The right internal jugular vein, the larger of the two, measured 3 cm. in diameter.

The liver weighed 2,150 Gm. and presented an irregularly nodular surface. It offered considerable resistance to cutting. Sections through it revealed nutmeg mottling. Microscopic examination revealed moderate to marked fibrosis, the bands of scar tissue radiating out from the portal spaces and fusing with those coming from adjacent portal spaces. Scattered chronic inflammatory cells were noted in the scarred areas. The spleen weighed 630 Gm. and was firmer than normal. Microscopic examination revealed congestion and a diffuse increase in fibrous tissue.

The other gross changes consisted in general visceral congestion and edema with 200 cc. of fluid in the abdomen, 200 cc. in the left pleural space and 800 cc. in the right. Microscopic examination confirmed the gross observations and revealed, in addition, numerous pigment-laden phagocytes in the alveoli of the lungs.

THE SPECIAL PATHOLOGY

The pathologic features of rheumatic disease of the tricuspid valve are those of marked congestive heart failure with, in addition, striking specific changes in the heart, veins and liver.

Heart.—Evidences of chronic mitral valvulitis are always found in the heart at autopsy; these changes are usually marked, having been found minimal only in the case described by Clements.³ The aortic valve is also severely involved in a majority of instances. The tricuspid valve is thickened and fibrosed and shows fusion of the cusps. This causes a considerable degree of valvular incompetence and also varying degrees of stenosis. The right auricle is enlarged, usually markedly so, so that its contents may exceed 2 liters.⁴ Changes in the wall of the right auricle are variable; in some instances hypertrophy has been noted, while in others marked thinning has occurred. The left auricle is usually dilated also, as a result of the mitral stenosis. Varying degrees of hypertrophy of both ventricular walls may be found; it is difficult, however, to evaluate the relative importance of the lesions of the various valves in its causation. On microscopic examination fibrotic changes and infiltration with a few round cells are found in the various valves, including the tricuspid. The myocardium may contain Aschoff nodules in various stages of evolution.

3. Clements, A. B.: *Am. J. M. Sc.* **190**:389, 1935.

4. Taussig, R. L.: *Am. Heart J.* **14**:744, 1937.

The pericardial fluid may be increased, or there may be a complete absence of the fluid, the latter occurring when the pericardium is completely adherent. The largest increase in pericardial fluid, 1,300 cc., recorded in case 1 of the present report, was associated with marked thinning of the right auricular wall. The pericardial effusion present in some of the cases reported must have caused cardiac tamponade.

Veins.—All the veins throughout the body are engorged and dilated; the most striking changes are found in both venae cavae and in the primary branches of the vena cava superior, which show an increase in diameter to three or four times the normal. Occasionally, localized dilatations may reach aneurysmal proportions. Fibrotic changes in the walls of the great veins have been described.⁵

Liver.—In most of the reports in which the liver is mentioned specifically it is described as fibrosed or cirrhotic. This change may result in a grossly nodular condition of the liver, although in other instances the gross changes are only minimal. It is of interest that on microscopic examination the type of fibrosis found is only occasionally central, or cardiac; more frequently it is recorded as portal in distribution. It has been noted previously that the incidence of portal fibrosis is abnormally high in patients with congestive heart failure.⁶ In patients in whom a considerable degree of cirrhosis is present, the spleen is enlarged and shows fibrotic changes post mortem. The ascites found in many patients with disease of the tricuspid valve is due, at least in part, to the cirrhotic changes in the liver.

PATHOLOGIC PHYSIOLOGY OF THE CARDIOVASCULAR SYSTEM

Studies⁷ were made of the circulatory dynamics during life in both the cases described in this article; in the first they were made before and after the onset of congestive heart failure, and in the second, during congestive failure and after a favorable response to therapy.

5. Futcher, T. B.: *Am. J. M. Sc.* **142**:625, 1911.

6. Altschule, M. D.: *Medicine* **17**:75, 1938. Katzin, H. M.; Waller, J. V., and Blumgart, H. L.: *Arch. Int. Med.* **64**:457, 1939.

7. All measurements were made under basal conditions with the patient in a semirecumbent position. The method of I. Starr and J. C. Gamble (*Am. J. Physiol.* **87**:450, 1928) was used for estimating the cardiac output; measurements of the respiratory dynamics were obtained at the same time. The venous pressure was measured in the antecubital and femoral veins by the direct method of F. Moritz and D. von Tabora (*Deutsches Arch. f. klin. Med.* **98**:475, 1910) using 18 gage or larger needles and short rubber connecting tubes. The capsule method of D. R. Hooker and J. A. E. Eyster (*Bull. Johns Hopkins Hosp.* **19**:274, 1908) was used to estimate the venous pressure in the veins over the dorsum of the hand. The arm to tongue circulation time was measured by means of sodium dehydrocholate as described by S. L. Gargill (*New England J. Med.* **209**:1089, 1933). Pulse beats and respirations were counted six to ten times during thirty second intervals.

The findings in both cases were very much alike and will therefore be treated together. When congestive heart failure was absent, the output of the heart was within the normal range of 1.4 to 2.1 liters per hundred cubic centimeters of oxygen consumed per minute. The venous pressure, however, was elevated in all the veins measured. The column of fluid in the manometer pulsated synchronously with the beat of the heart, the extent of the pulsation varying between 0.4 and 2.0 cm. of water. The pulsations in the femoral veins were about twice as large as those in the antecubital veins. The values recorded in table 2 are those obtained in diastole. Because of the presence of auricular fibrillation, the duration of diastole varied greatly; over a period of a few

TABLE 2.—Measurements of Cardiovascular and Respiratory Function in Two Cases of Rheumatic Disease of the Tricuspid Valve

	Case 1		Case 2	
	Before Failure	During Failure	During Failure	After Treatment
Venous Pressure, {				
Cm. of {				
Water {				
Arm.....	17.22	35	14	12
Hand.....	21
Femora.....	22	..	22	..
Cardiac output, liters per minute.....	2.94	2.25	4.43	5.28
Cardiac output, liters per hundred cubic centimeters of oxygen consumed.....	1.56	1.13	1.31	1.75
Arteriovenous oxygen difference, volumes per cent	6.4	8.9	7.7	5.7
Pulse beats (average rate) per minute.....	68	50	76	76
Cardiac stroke volume per cubic centimeter.....	47	28	58	70
Circulation time, seconds.....	25	28	37	23
Respirations per minute (average rate).....	12.5	16	16	14
Respiratory volume, liters per minute.....	4.8	5.4	7.4	7.1
Tidal air, cubic centimeters.....	384	337	462	523
Vital capacity, cubic centimeters.....	1,400-1,600	600-900	1,700	1,800
Oxygen consumption, centimeters per minute....	189	200	339	305
Basal metabolic rate, per cent.....	-3	+2	+39	+28
Alveolar carbon dioxide, per cent.....	4.1	..	5.2	5.2
Respiratory quotient	0.84	0.83	0.82	0.82

minutes it was possible to obtain readings during periods of diastole of varying length. The length of diastole did not influence the readings; the column of fluid in the manometer came to a standstill at the same point each time.

The finding of persistently elevated venous pressures throughout the body with a normal cardiac output is to be expected when there is some obstruction to the heart's inflow of blood. The obstruction was the stenosis of the tricuspid valve, which caused a damming up of inflow until a sufficient head of pressure developed to maintain passage of normal amounts of blood. The elevated venous pressure in such a situation is necessary for the maintenance of the normal cardiac output; lowering it, by venesection for instance, might cause shock or even death. The additional increase in venous pressure during systole was due to regurgitation of blood from the ventricle through the incompetent valve.

Both patients were free of edema in spite of the elevated venous pressure at the time the described measurements were made. It has been pointed out elsewhere⁹ that the occurrence of edema is due to the action of a multiplicity of factors and that only rarely do extreme changes in a single factor cause development of edema. It failed to develop at this time in both cases, probably because the cardiac output and plasma protein levels were normal.

In spite of the fact that the cardiac output was normal and that there were no signs of congestive heart failure at the time of study the blood flow was found to be somewhat slowed. This may have been due mainly or entirely to the slowing of the blood flow in the venous portion of the circulation. The diminution of vital capacity observed at this time was probably due to the encroachment of the tremendous enlargement of the heart on the pulmonary space. The decrease in available respiratory space probably contributed to the dyspnea in these patients.

The fact that when the patients were free of congestive failure the cardiac output at rest was normal is no indication that the response to exercise would have been normal. Indeed, the fact that dyspnea appeared in both patients on exertion for many years before the onset of frank congestive heart failure suggests that the lesion of the tricuspid valve would not permit the increased inflow into the heart which normally occurs during exercise, so that the resulting cardiac output was lower than normal for that level of activity.

The observations on the blood gases are fragmentary. In case 1 blood obtained from the femoral vein before the onset of frank congestive heart failure revealed an oxygen content of 13.1 volumes per cent and a saturation of 67.2 per cent. Both of these figures are within normal limits. This finding together with the normal values for arteriovenous oxygen difference obtained at the time of the measurements of the cardiac output indicates that the cyanosis which was noted in the absence of frank congestive heart failure was not due to stagnation but rather to marked general venous and venular engorgement. This type of cyanosis has been produced experimentally by Goldschmidt and Light.¹⁰

Observations made while the patients showed signs of congestive heart failure revealed conditions similar to those in other decompensated cardiac patients.⁶ The cardiac output was lower, the arteriovenous oxygen difference was increased, the blood flow was slower and the venous pressure was increased above the level found when the patient was not decompensated. Associated with these changes in cardiovascular dynamics were the expected increases in respiratory rate and minute volume and in basal metabolic rate. The respirations were

8. Footnote has been deleted.

9. Altschule and Blumgart.² Altschule.⁶

10. Goldschmidt, S., and Light, A. B.: *Am. J. Physiol.* **73**:173, 1925.

shallower than normal, as shown by the decrease in tidal air. The vital capacity was diminished. At the time these studies were made, moderate edema was present, and dyspnea, cyanosis and orthopnea were greater than when the patient was not grossly in cardiac failure. Later all these signs and symptoms increased; it is to be assumed that the changes in cardiovascular and respiratory dynamics likewise progressed. The disproportionately high venous pressures as well as the other changes in cardiovascular dynamics usually found in decompensated patients with disease of the tricuspid valve are similar to those occurring in constrictive pericarditis.¹¹

THE CLINICAL SYNDROME

The clinical findings in our 2 patients are similar to those reported by other authors.¹ There is usually a history of a childhood period of rheumatic fever or chorea, followed by a number of years during which cardiovascular symptoms were absent. Then, most often late in the second or in the third decade, dyspnea on exertion develops, with or without mild orthopnea. At this time engorgement and pulsation of the veins of the neck may be noted. There is usually little or no progression of symptoms in spite of a fairly active life for some years. This suggests that the early exertional dyspnea is not due to progressive myocardial weakness but rather to a mechanical obstruction preventing the increases in cardiac output required during exercise. Some patients may have attacks of dizziness or syncope. Later, when congestive heart failure develops, dyspnea, orthopnea and venous engorgement increase, and marked edema and ascites appear. The marked edema of the patient with disease of the tricuspid valve usually responds poorly to treatment. In many cases ascites develops early and is the most troublesome symptom. In such instances abdominal paracentesis must be performed repeatedly because of recurrent ascites and the course at first is more that of cirrhosis of the liver than that of congestive heart failure.¹² The patients complain chiefly of dyspnea, anorexia and weakness, all of which are greatly relieved by paracentesis; cough, orthopnea and edema may be minimal. The spleen in such cases is palpable during life. Indeed, in such cases marked cirrhosis of the liver is found at postmortem examination. Ultimately, however, in these cases, too, the heart fails, and very marked edema, cyanosis, orthopnea and dyspnea develop.

11. Beck, C. S., and Griswold, R. S.: *Arch. Surg.* **21**:1064, 1930. Burwell, C. S., and Strayhorn, W. D.: *ibid.* **24**:106, 1932. Beck, C. S.: *J. A. M. A.* **102**:1543, 1934. Burwell, C. S., and Flickinger, D.: *Arch. Int. Med.* **56**:251, 1935. Griswold, R. A.: *J. A. M. A.* **106**:1054, 1936. Stewart, H. J.; Hener, G. J.; Deitrick, J. E.; Crane, N. F.; Watson, R. F., and Wheeler, C. H.: *J. Clin. Investigation* **17**:581, 1938.

12. Thompson, W. P., and Levine, S. A.: *Am. J. M. Sc.* **193**:4, 1937.

Physical examination reveals, in addition to the usual manifestations of congestive heart failure and cirrhosis of the liver, certain specific signs which point to the diagnosis of organic disease of the tricuspid valve. The color of the skin is striking. It can best be described as a mixture of cyanosis and mild icterus.¹³ The icterus is caused by stasis and is due both to impaired function of the liver and to increased destruction of red blood cells; the occurrence of the latter is proved by the presence of anemia and reticulocytosis. There is marked engorgement of the veins, particularly noticeable in the neck. Not only are the veins full, but they are increased in diameter two to four or more times. Retrograde pulsations due to the insufficiency of the tricuspid valve are a striking finding. These pulsations may be diphasic, consisting of a presystolic (auricular) and a systolic (ventricular) component. In patients with auricular fibrillation the presystolic component is, however, absent. The heart is enlarged bilaterally to a marked degree. In addition to the murmurs from a diseased mitral valve, which all patients show, and those from a diseased aortic valve, which many exhibit, other murmurs are heard to the right of the lower part of the sternum and the xiphoid process. These can usually be distinguished from those accompanying disease of the mitral valve by differences in quality. The cardiac rhythm is frequently grossly irregular because of auricular fibrillation; other arrhythmias may, however, occur.¹² The liver is enlarged, usually markedly, and pulsates synchronously with the veins in the neck. Pressure on the liver increases the engorgement of the veins in the neck. Late in the course of the disease, as fibrosis progresses, the liver may become smaller and cease to pulsate. At this time and sometimes earlier the spleen can be felt also. Two reports of pulsation of the spleen in disease of the tricuspid valve are available.¹⁴ In one instance^{14a} the splenic pulsation was synchronous with the arterial rather than with the venous pulse. It is, however, impossible to describe the special anatomic feature responsible for this phenomenon since postmortem examination was not made in either instance.

Edema at first involves the legs only and may become quite pronounced there. Later, however, it becomes general, although still most marked over the legs and sacrum. Treatment of this edema is unsatisfactory because of the persistently high venous pressures and also the low levels of serum protein which ultimately develop. The latter fault is due to malnutrition, albuminuria and the loss of large amounts of protein from the body through frequent abdominal paracenteses.

13. Wearn, J. T., in *Medical Papers Dedicated to Henry Asbury Christian*, Baltimore, Waverly Press, Inc., 1936, p. 60.

14. (a) Manger, M.: *Am. J. M. Sc.* **154**:72, 1917. (b) Sutton, D. C., and Rawson, V.: *Am. Heart J.* **10**:1096, 1935.

Late in the course of the disease purpura may occur, usually over the legs. The mechanism responsible for the appearance of this sign has not been studied, but it appears that the persistently high venous pressure may be implicated. Many patients with chronic diseases have subclinical scurvy; it is possible that the additional factor of high venous pressure that is found in disease of the tricuspid valve serves to make it overt.

The engorgement of the veins of the face which develops in patients with disease of the tricuspid valve makes it possible to study the role of cerebral venous distention in the genesis of orthopnea. In a patient so studied² it was found that lowering the head until the veins became engorged to about the level of the respiratory center caused orthopnea.

A small pulse has been described as occurring in disease of the tricuspid valve, but was not noted in the 2 cases here reported. Its occurrence in other cases is difficult to evaluate since aortic stenosis, which is known to cause a small pulse, is frequently found in instances of rheumatic disease of the tricuspid valve.

It was noted in a foregoing paragraph that the cardiovascular dynamics in disease of the tricuspid valve are much like those of adhesive pericarditis. It has been pointed out that the clinical features of the two syndromes are also similar;¹⁵ this may lead to an erroneous diagnosis. Nevertheless, if the typical murmurs from a diseased tricuspid valve are sought for, the correct diagnosis will usually be made.

The temporary relative insufficiency of the tricuspid valve associated with severe congestive heart failure¹⁶ is easily differentiated from the organic disease. In the former the venous and hepatic pulsations are less marked, the veins are distended but not so markedly increased in diameter, and the typically situated murmurs are absent. Moreover, all the signs disappear with improvement.

The clinical features of congenital disease of the tricuspid valve are much like those of the rheumatic disease. However, certain important differences exist. Associated with the former there are usually other congenital cardiac lesions, such as congenital pulmonic stenosis, with its typical signs or septal defects, which cause early cyanosis and clubbing of the fingers. In the congenital type of insufficiency the signs of severe disease of the mitral and aortic valves are absent and auricular fibrillation does not occur.

In our own experience the clinical findings in a case of congestive heart failure due to an auricular septal defect and those in a case in which venous stasis was due to marked general mediastinal inflammatory

15. Mackenzie, J.: *Diseases of the Heart*, London, Hodder & Stoughton, 1908. Altschule.² Thompson and Levine.¹²

16. Kerr, W. J., and Warren, S. L.: *Arch. Int. Med.* **36**:593, 1925.

fibrosis were similar to those of disease of the tricuspid valve except for the typical murmurs of the latter. The true diagnoses were made clinically and proved correct by postmortem examination.

SUMMARY

The clinical syndrome of rheumatic disease of the tricuspid valve is characterized by distention and increase in diameter of all visible veins, hepatomegaly, systolic (and in the absence of auricular fibrillation pre-systolic) venous and hepatic pulsations, cyanosis, jaundice, enlargement of the heart to the right and murmurs over the area of the tricuspid valve. The clinical evidences of cirrhosis of the liver or of congestive heart failure may complicate the picture. The special pathologic condition causing this syndrome consists in insufficiency and variable degrees of stenosis of the tricuspid valve, dilatation of the right auricle and of the veins and cirrhosis of the liver. The cardiac output is normal when congestive failure is not present. Nevertheless, the venous pressure is elevated, proving that the rise in venous pressure is due to mechanical obstruction to the heart's inflow of blood. When cardiac decompensation occurs, the output of the heart diminishes and the venous pressure rises above its former high level. The relation of the pathologic and physiologic features of the syndrome to its clinical manifestations is discussed in detail.

RENAL LESIONS ASSOCIATED WITH DEEP JAUNDICE

WITH COMMENTS ON THEIR RELATIONS TO THOSE IN THE
SO-CALLED HEPATORENAL SYNDROME AND IN TRANS-
FUSION REACTIONS

DARRELL AYER, M.D.

BOSTON

Fatal anuria has been observed frequently following operations on the biliary passages (hepatorenal syndrome) and following transfusions of incompatible blood. Examination of kidneys from patients who died of these two conditions has demonstrated pigments,¹ such as are produced by the breakdown of hemoglobin, in the tubules and degeneration of the tubules.² In order that these findings might be interpreted properly, it was necessary to ascertain the extent of the renal lesions associated with persistent biliary obstruction in man. So as to avoid the possibility of other factors, i. e., chronic cholecystitis, neoplasm, arteriosclerosis and vascular nephritis, having any part in the production of these changes, it seemed advisable to limit this preliminary investigation to observation of the renal lesions in a series of infants with congenital atresia of the bile ducts.

The autopsy material from 18 infants who died of congenital atresia of the bile ducts was used for this study. This material represented all autopsies on infants with that condition made between January 1924 and December 1938. For microscopic examination, blocks of kidney fixed in Zenker's fluid and in solution of formaldehyde U. S. P. diluted 1:10 were available. The general descriptions were made using sections fixed in Zenker's fluid and stained with hematoxylin and eosin. Scarlet red, hematoxylin and von Kossa stains were done on formaldehyde-fixed material. Phosphotungstic acid-hematoxylin stain, Mallory's aniline blue stain and the Turnbull blue iron stain were used in selected cases.

First, the renal changes observed microscopically were recorded. Following this, the clinical records and the complete autopsy reports were analyzed in an

From the Department of Pathology, Harvard Medical School.

1. This vague term is used here to include the iron-free bile and blood pigments and the iron-containing hemoglobin, because in fixed material no specific stains for the iron-free pigments are applicable and the iron in hemoglobin is too intimately bound to the protein group in the molecule to react to the ordinary tests (Mallory, F. B.: *Pathological Technique*, Philadelphia, W. B. Saunders Company, 1938, p. 135).

2. Bell, E. T.: *Am. J. Path.* **13**:497, 1937. Helwig, F. C., and Orr, T. G.: *Arch. Surg.* **24**:136, 1932.

attempt to discover some correlation between the renal changes and such incidental factors as infections, transfusions, hemorrhages and operations. Jaundice was the only clinical finding common to all the cases. A summary of the clinical and general postmortem observations will be given, followed by a more detailed description of the kidneys.

The cases (table 1) are arranged in order of the ages of the patients at the time of death. The ages, and thus the duration of jaundice, varied from 23 to 374 days. In 16 infants exploratory operations disclosed inoperable atresia of the bile ducts. Death occurred from twenty-five minutes to thirty-three days after the operation. Three patients became eviscerated. In 2 of these fatal peritonitis developed; the third died of shock. Clinical evidences of infection are given in table 1. The autopsy observations and the causes of death are recorded in table 2. All the patients showed some degree of dietary deficiency, but only the morphologic changes of vitamin A deficiency³ are noted in the table. Four infants died of hemorrhage and 8 of infection, but the chief cause of death in the majority of the series was nutritional disorder. However, when even the slightest infection was found, the infection is listed in the cause of death column, and nutritional disorder is entered as a cause of death only in the cases without infection. No patient had been exposed to mercury or other heavy metal. Since neither vitamin A deficiency nor infection was a constant finding, and since several patients had neither bleeding nor transfusion, jaundice remained the clinical feature common to all.

On macroscopic examination all the kidneys were deeply jaundiced. Otherwise they were essentially normal. Even the few with pyelitis or the changes of vitamin A deficiency microscopically showed no lesions macroscopically. Six showed slight engorgement of blood vessels, but there was no gross evidence of edema. Microscopically, the involved kidneys were characterized by the presence of pigmented casts in the distal convoluted and collecting tubules and by changes in the epithelium and in the connective tissue surrounding these cast-filled tubules. The severity of these lesions bore no constant relation to the duration of the jaundice. For the sake of convenience in description, the cases are divided into four groups on the basis of the extent and severity of the renal changes.

In group 1 are the cases with heavy polymorphonuclear leukocytic infiltration of the cast-filled distal convoluted and collecting tubules and about these the most severe exudative reaction, consisting of edema and leukocytic infiltration of the interstitial tissue. In group 2 are the cases with leukocytic infiltration confined to the cast-filled tubules. About these tubules there is slight edema of the connective tissue, but there is no cellular infiltration. In group 3 there is neither edema nor

3. Blackfan, K. D., and Wolbach, S. B.: *J. Pediat.* **3**:679, 1933.

TABLE 1.—Clinical Data

Case	Sex	Age and Duration of Icterus, Days	Urine			Icterus Index	Transfusion			Infection			
			Bile	Albumin	Sediment		Edema	Number	Last Before Death, Days	Opera- tion, Days Before Death	Type	Duration	Days Before Death
1	M	23	None	None	None	..	2	None
2	M	39	300	+	Many granular casts	None	None	None	..	19	None
3	M	46	None	None	11	None
4	F	61	None	Right side of face and both eyes	None	..	1/4	None
5	F	64	130	+	Rare white blood cells	None	None	None	..	19	Diarrhea	4 days	4
6	M	77	None	None	1	4	1	None
7	F	86	50	+	Amorphous debris...	None	None	1	22	22	Stitch abscesses, bronchopneumonia	To death	21
8	M	91	75	+	None	None	None	1	8	8	Peritonitis	8 days	8
9	M	102	50	+	Occasional red blood cells	None	None	1	Patient died during it	No opera- tion	Broncho pneumonia	7 days	7
10	M	117	70	+	None	None	None	1	7	7	None	Eviscerated
11	F	140	125	+	Many hyaline casts...	None	None	3	5	10	Peritonitis- meningismus	3 days	Eviscerated
12	F	174	180	+	None	None	None	None	..	9	Otitis media	7 days	50
13	F	179	60	+	None	None	None	None	..	25 minutes	Acute peritonitis	3 days	Eviscerated
14	F	184	325	+	None	None	Ascites (9 days)	None	..	1/2	None
15	F	196	120	+	None	None	None	None	..	14	? Bronchopneumonia	1 day	1
16	M	214	225	+	Rare white blood cells	None	Legs (30 days) Ascites (9 days)	2	60	(1) 60	Bronchopneumonia	14 days	14
17	M	238	None	None	(3) 33
18	F	374	...	+	None	None	None	1	1	No opera- tion	Temperature, 105 F. Otitis media	1 day 10 days	1 154

TABLE 2.—Pathologic Observations

Distal Convoluted Tubules and Collecting Tubules of Cortex																	
Case	Weight of Kidneys, Gm.	Stromal Reaction	Epi- thelial			Epithelium of Henle's Loops			Collecting Tubules of Medulla		Glomeruli		Vita- min A Defec- tency	Liver		Cause of Death	
			Leuko- cytes	Dila- tation; Phago- cytosis	Emphy- sema	Fat	Pig- ment	Fat	Casts	Dilatation	Hyaline Fibrosis of Loops	Dilatation		Pyc- nolysis	Biliary Cirrhosis		Focal Necrosis
1	30 (together)	+	90%	++	Rare	5%	+	+	+	33%	All	None	All	None	+	None	Hemorrhage into peritoneum and intestine
2	R. 8, L. 16	+	90%	++++	Rare	5%	+	+	+	33%	90%	Rare	All (sl.)	None	+	None	Hemorrhage into many organs
3	R. 7, L. 7	+	90%	+++	Occa- sional	5%	+	+	+	10%	All	Rare	All (sl.)	None	+	None	Septicemia (Streptococcus haemolyticus); hemorrhage into intestine
4	Not weighed	+	25%	+	Rare	5%	+	+	+	0	All (sl.)	None	All (v.sl.)	None	+	None	Nutritional disorder; post-operative shock
5	R. 18, L. 18	+	50%	+++	Fre- quent	80%	+	+	+	0	All	None	All (v.sl.)	None	+	None	Nutritional disorder; bronchopneumonia
6	Not weighed	+	50%	+	Fre- quent	2-3%	Not done	+	+	10%	50%	None	All (v.sl.)	None	+++	+	Nutritional disorder; post-operative shock
7	R. 14, L. 14	+	25%	+	Occa- sional	50%	+	+	+	50% (sm.)	All (sl.)	None	All (v.sl.)	None	++	None	Bronchopneumonia
8	R. 14, L. 12	+	90%	+	Rare	All (sl.)	+	+	+	0	All (v.sl.)	Rare	All (sl.)	None	+	None	Localized peritonitis; terminal bronchopneumonia
9	R. 12, L. 12	None	10%	Very rare	Occa- sional	90%	0	+	+	0	All (sl.)	Rare	All (sl.)	None	+	None	Acute bronchopneumonia
10	R. 26, L. 28	None	10%	Rare monocyte	Fre- quent	30%	+	+	+	0	All (sl.)	None	All (sl.)	+	++	None	Shock and acute pyelitis
11	R. 22, L. 28	+	10%	+	None	50%	+	+	+	0	All (sl.)	Rare	All (sl.)	None	+	None	General sepsis
12	R. 33, L. 36	None	Rare	None	None	All (sl.)	+	+	+	0	All (sl.)	None	All (sl.)	+	++	None	Acute peritonitis; bronchopneumonia
13	R. 36, L. 35	None	Rare	None	None	All (sl.)	+	+	+	0	All (sl.)	None	All (sl.)	None	+	None	Nutritional disorder; shock
14	R. 42, L. 38	None	Rare	None	None	All (sl.)	+	+	+	0	All (sl.)	None	All (sl.)	None	+	None	Hemorrhage into lungs
15	R. 23, L. 25	None	10%	None	Rare	50%	+	+	+	5%	All (sl.)	None	All (sl.)	None	+++	None	Nutritional disorder
16	R. 36, L. 38	None	5%	None	Occa- sional (mod.)	All (mod.)	+	+	+	0	All (mod.)	None	All (sl.)	None	++	None	Bronchopneumonia, slight
17	R. 46, L. 53	None	90%	None	Occa- sional	50%	+	+	+	Rare	All (sl.)	None	All (sl.)	None	+++	None	Bronchopneumonia, slight
18	R. 28, L. 28	+	80%	+	Fre- quent	Occa- sional	+	+	+	Rare	All (sl.)	None (increased cells only)	All (sl.)	None	+	None	Hemorrhage into intestine

cellular infiltration of the interstitial tissue. The casts are infiltrated by monocytes alone and in small numbers. In group 4 all of the preceding changes are absent except for occasional pigmented casts in the distal convoluted tubules.

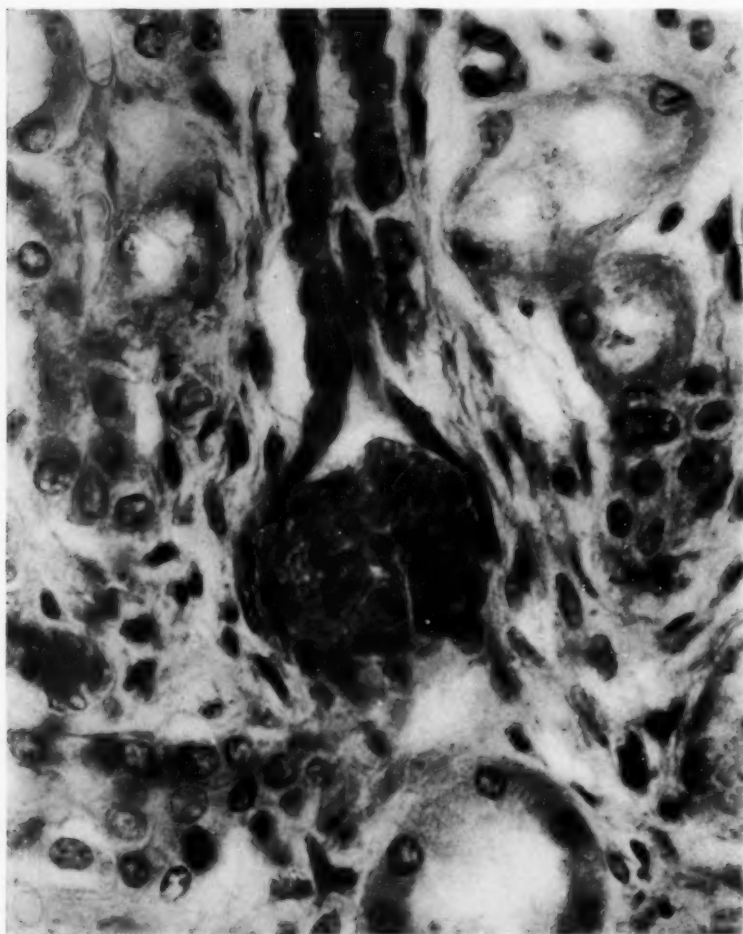


Fig. 1.—A collecting tubule (case 2) showing flattening of the epithelial cells in the portion distended by the cast; $\times 900$. There are also slight edema and proliferation of connective tissue about this portion.

CHANGES IN THE KIDNEYS

Group 1 (Cases 1, 2, 3, 4, 5, 6 and 18).—Most of the distal convoluted and many of the collecting tubules were distended with pigmented casts. The majority of the cast-filled tubules were lined by well

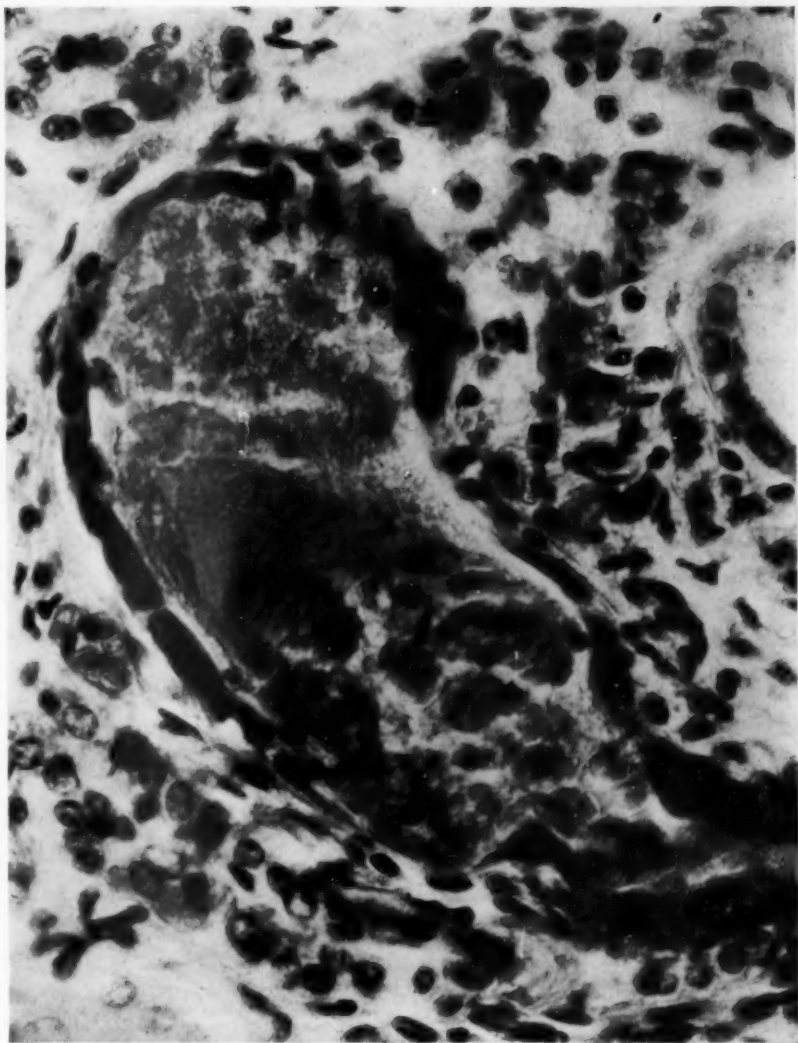


Fig. 2.—Distal convoluted tubule (case 2); $\times 900$. In the upper portion of the lumen lies loosely packed pigmented granular material; in the middle portion, a more tightly packed form, and in the lower part, discrete rounded masses. No cellular infiltration is seen. To the right of this tubule is a small mass of pigmented cast material surrounded by desquamated epithelial cells and leukocytes, representing the site of a tubule.

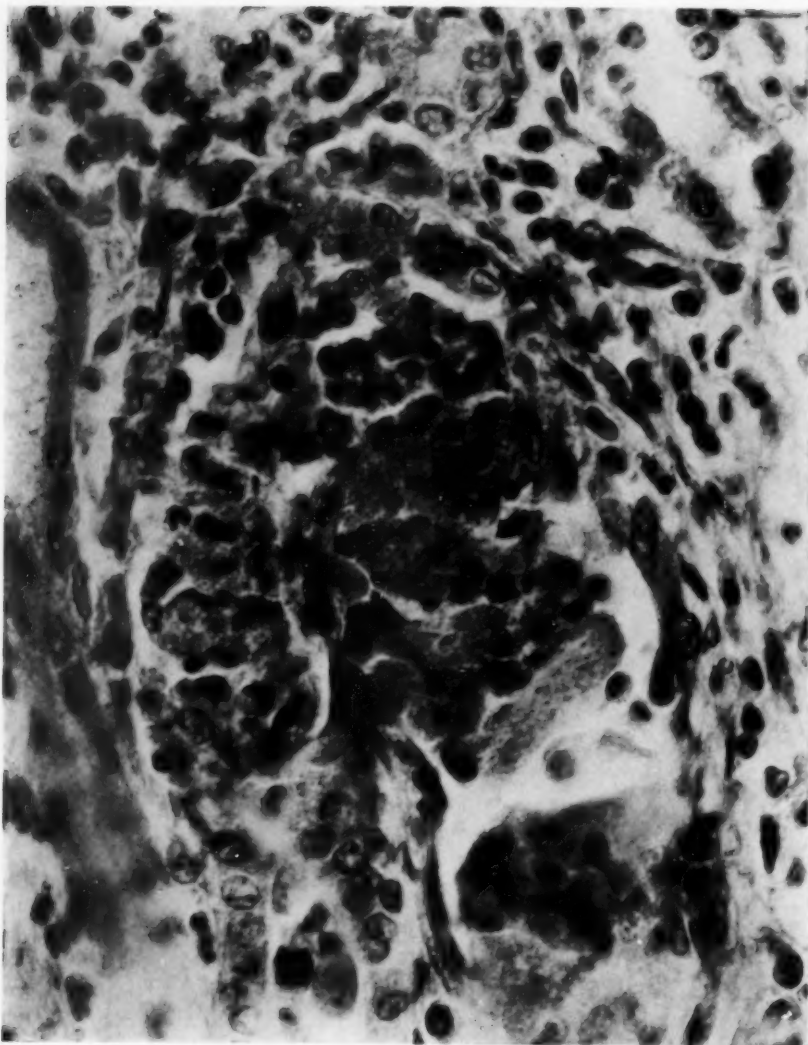


Fig. 3.—A distal convoluted tubule (case 2) showing fragments of cast material infiltrated by leukocytes; $\times 900$. In the upper right part the epithelium is desquamated and mixed with monocytes. Above the tubule leukocytes lie in the interstitial tissue.

preserved cells, some flattened, some with pyknotic nuclei (figs. 1 and 2). The slightest evidence of cellular reaction to the cast material was found in the occasional cast-filled tubule that contained one or more monocytes in the epithelium or in the cast. Less frequent but numerous were the tubules containing many monocytes and polymorphonuclear leukocytes, which had surrounded and engulfed bits of the cast material (fig. 3). About some such tubules there were signs of edema; about others there was slight increase in the number of connective tissue cells. More severe changes consisted of heavy infiltration of this surrounding connective tissue with monocytes, polymorphonuclear leukocytes, lymphocytes and eosinophils. When desquamation of the tubular epithelium was associated with leukocytic infiltration of the cast and the surrounding connective tissue, the resulting lesion suggested a stage in the removal of cast material and dead tubular epithelium in preparation for repair by cicatrization (figs. 2 and 3). However, there were no real cicatrices. Other bits of cast material were handled in another way. Some of the cast material was phagocytosed by epithelial cells. In some tubules the entire cast was covered by a thin rim of the cytoplasm of a single cell with a slender nucleus that curved to fit the cast. Occasionally, a large multinucleated epithelial cell, remaining attached to the basement membrane, incorporated a rounded mass of the pigmented cast material (fig. 4).

The casts were usually composed of granular material, with the particles slightly larger than those of the familiar granular debris of the proximal convoluted tubules. For the most part the granules had a brown or greenish brown color of varying intensity. Occasionally, they were bright red or faintly reddish brown. This material was rather loosely packed in some tubules; in others, so tightly packed as to approach a homogeneous appearance. In other tubules it was arranged in round masses the size of cells but lacking nuclei (fig. 2). Occasionally, bits of pale acidophilic homogeneous material, resembling ordinary hyaline casts, were mixed with the granular material.

The proximal convoluted tubules contained only circular reticulum (fig. 5) and finely granular acidophilic material. There were no casts or pigment. All were slightly dilated; the cells were all low and the nuclei well preserved.

Cells of Henle's loops contained extremely fine, pale yellow granules which did not show iron on staining. These were more numerous in the descending limb. These tubules were not dilated and contained no casts.

The collecting tubules of the cortex contained casts and showed the same types of infiltration as the distal convoluted tubules. Degenerative changes, edema and polymorphonuclear leukocytic infiltration were

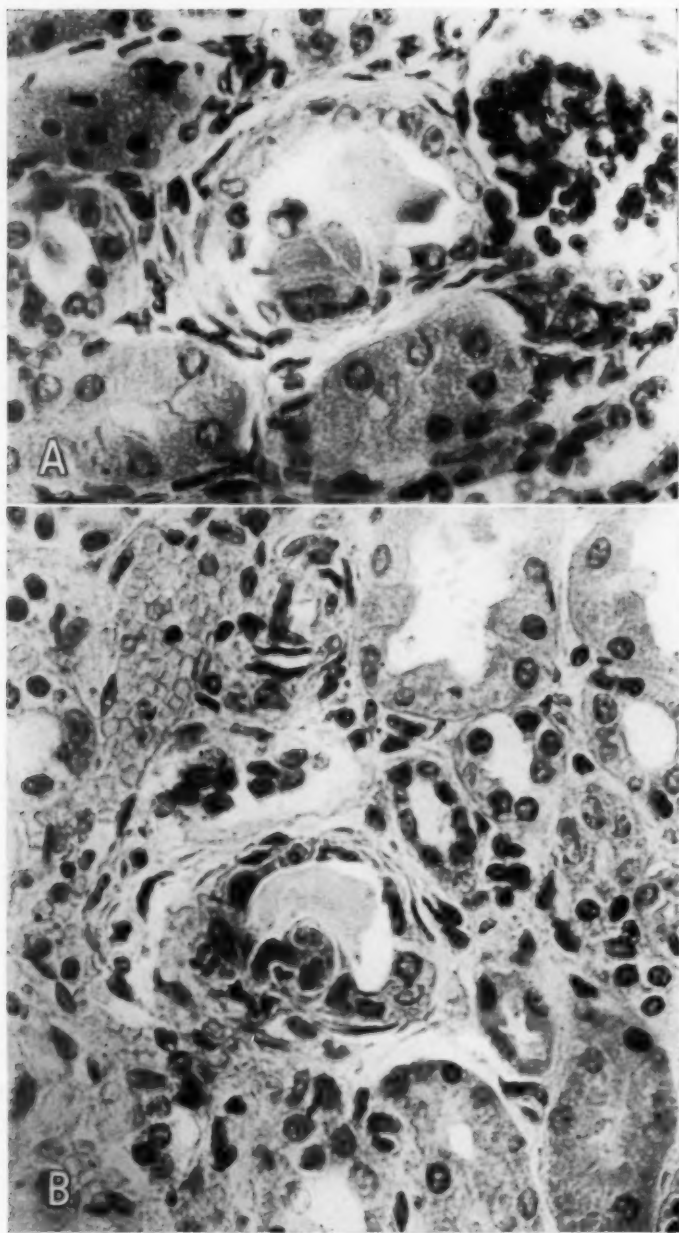


Fig. 4.—*A*, distal convoluted tubule (case 5) in which a large epithelial cell, with two separate elongated nuclei at the base, contains slightly pigmented acidophilic material, separated into a large and a small mass by an oblique fissure; $\times 500$. To the right of the smaller mass of pigmented material can be seen a slender prolongation of the nonpigmented cytoplasm of the cell.

B, epithelial cells of a distal convoluted tubule (case 10) containing pigmented acidophilic material; $\times 500$. The largest mass, shaped like a kidney with the hilus turned to the left, is surrounded by a narrow clear zone. In the portion projecting into the lumen this clear zone is covered by slender projections of the cytoplasm of adjacent epithelial cells. To the left of these cells is a single epithelial cell with pigmented material lying deep to the nucleus.

particularly marked about many of these tubules at the zone of junction of the cortex and medulla (case 2). Farther down in the pyramids there was no edema or cellular infiltration. In cases 1 and 2 approximately a third of the large collecting tubules were greatly distended with casts without any accompanying leukocytic infiltration into the casts or around the tubules (fig. 6). No mitotic figures were seen.

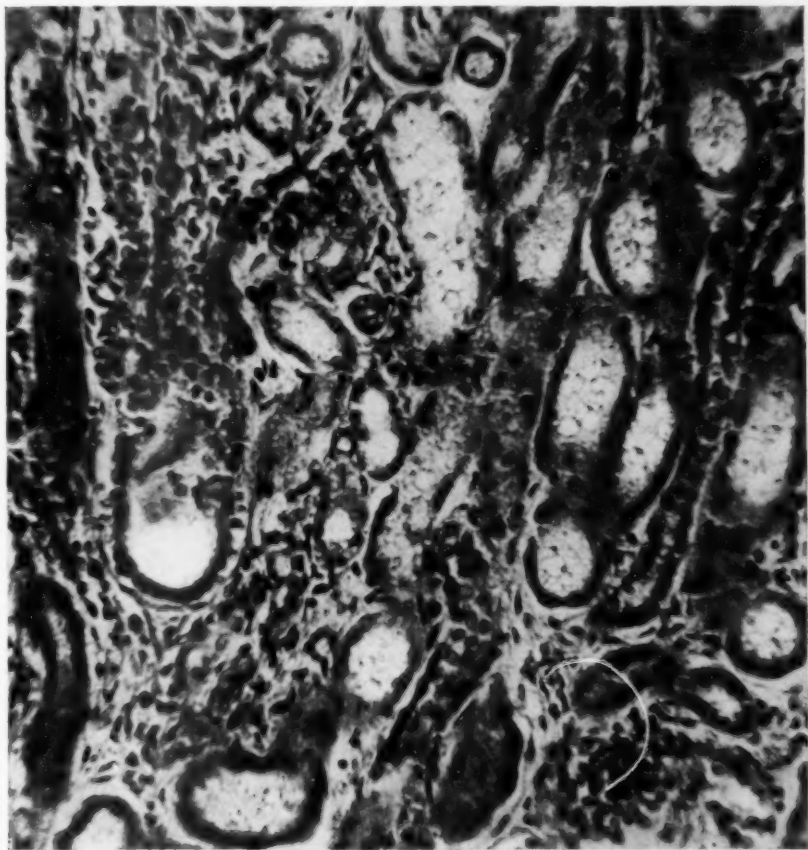


Fig. 5.—To the lower left a cast-containing dilated collecting tubule (case 2) is surrounded by edema and scattered infiltrating cells; $\times 300$. In contrast, the proximal convoluted tubules in the upper right lie close to one another and contain only circular reticulum.

In cases 2 and 3 a rare glomerulus showed a single loop composed of a hyalinized fibrous center, covered on the surface by epithelial cells, but there were no observable sequences to explain such structures. The other loops of such glomeruli and all other glomeruli were normal. All

showed slight dilatation of the capsular spaces and rare fine granules of acidophilic debris.

The stroma showed no diffuse edema or cellular infiltration. There were areas of focal edema or focal infiltration corresponding to the changes in the distal convoluted and collecting tubules. The blood vessels were normal. In case 2 rare free monocytes in the interstitial tissue contained hemosiderin, demonstrable by iron stain.

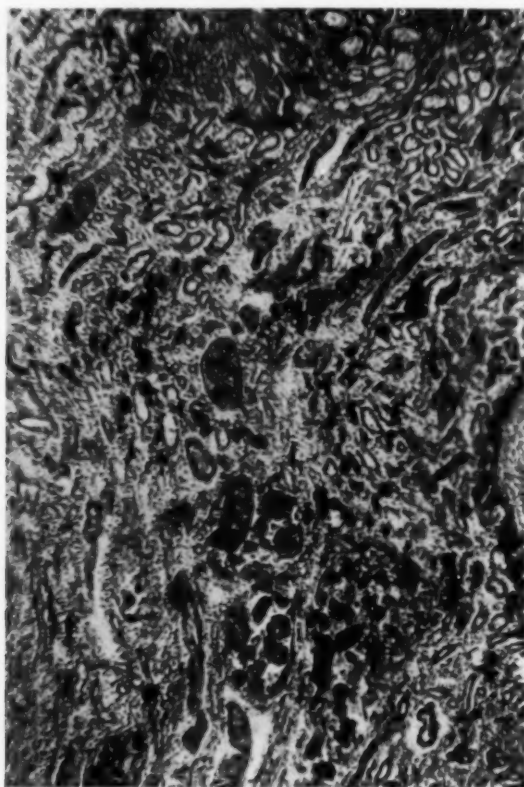


Fig. 6.—The zone of contact of cortex and medulla showing collecting tubules distended with casts (case 2); $\times 65$.

With the scarlet red stain, small fat droplets were found in the cells of Henle's loops and in those of some of the distal convoluted and collecting tubules.

With Gram and Giemsa stains, sections showed no micro-organisms. No calcium was demonstrable in sections stained by von Kossa's method.

Group 2 (Cases 7, 8 and 11).—The kidneys in this group differed from those in group 1 in the absence of cellular infiltration of the stroma,

the smaller number of cells infiltrating the casts and the smaller number of cast-filled tubules. Only about one fourth of the distal convoluted tubules and collecting tubules of the cortex contained casts. Occasional casts were infiltrated by small numbers of monocytes and polymorphonuclear leukocytes. About these tubules there was evidence of very slight edema, with increased prominence of the connective tissue cells, but there was no cellular infiltration. The proximal convoluted tubules contained only circular reticulum. All of the proximal convoluted tubules, about one half of the empty distal convoluted tubules and the glomerular capsules were slightly dilated. Rare glomeruli in cases 8 and 11 showed hyalinized loops. In the distal convoluted tubules there were occasional examples of inclusion of bits of pigmented necrotic material within the cytoplasm of epithelial cells, some of which were multinucleated. No cicatrices were present in the cortex or medulla. There was no evidence of infection. The vessels were normal. In the scarlet red stains fat droplets were demonstrated in the cells of the distal convoluted tubules, both limbs of Henle's loops and many of the casts. There was no calcium. In the iron stains (case 7) rare hemosiderin-laden macrophages lay in the interstitial tissue, but there was no iron in any of the epithelial cells.

Group 3 (Cases 9, 10, 15, 16 and 17).—The kidneys of this group were characterized by the complete absence of evidence of edema or cellular infiltration of the interstitial tissue. There were fewer casts in the distal convoluted and collecting tubules of these kidneys than in those of group 2. Only extremely rare monocytes were seen infiltrating a rare cast. Epithelial phagocytosis of necrotic material was present in all kidneys of this group and were particularly numerous in those of case 10 (fig. 4B). No mitotic figures were seen in the tubules. A rare glomerulus in case 9 contained a single hyalinized loop, but the remaining glomeruli in this case and all glomeruli in the other cases were normal except for very slight dilatation of the capsules. Pigment and fat droplets were present in the cells of Henle's loops. Calcium and iron stains were negative. The vessels were normal. Case 10 was of particular interest because there was heavy infiltration of the pelvis with polymorphonuclear leukocytes. Cultures of the pelvis yielded growths of *Bacillus coli* and *Staphylococcus aureus*. Despite this infection in the pelvis, the kidneys showed no edema or cellular infiltration of the interstitial tissue, and the tubules and casts contained no polymorphonuclear leukocytes. In fact, the kidneys showed as little change as any in the group.

Group 4 (Cases 12, 13 and 14).—In these kidneys there were extremely rare pigmented casts in the distal convoluted tubules. There were pale pigment and fat droplets in the cells of Henle's loops and fat alone in those of the distal convoluted tubules. All the tubules and

glomerular capsules were very slightly dilated. All the other changes described in the preceding groups were absent. In case 12 there was marked squamous metaplasia of the pelvic epithelium, with heavy polymorphonuclear leukocytic infiltration in and beneath this epithelium. Despite this evidence of pyelitis and vitamin A deficiency, the kidneys showed no cellular infiltration in or about the tubules and only the rarest small cast.

CHANGES IN THE LIVERS

All the livers were deeply jaundiced, enlarged and showed evidence of progressive biliary cirrhosis. The slightest degree of change consisted of marked bile stasis and early evidence of the invasion of liver cells at the periphery of the lobule by polymorphonuclear leukocytes, but there was no connective tissue increase or bile duct proliferation (cases 1, 14 and 18). The most severe degree of fibrosis and bile duct proliferation was found in cases 15 and 17. In case 6 there were no normal liver cells; each lobule was made up of syncytial masses of regenerating liver cells. Small focal necroses were found in cases 2 and 6. Marked fatty metamorphosis was present in cases 3, 6 and 9. There was no relation in severity between the lesions of the liver and those of the kidneys.

SUMMARY OF PATHOLOGIC OBSERVATIONS

The one constant feature of all cases was the presence of casts containing essentially iron-free brown pigment. These casts occurred principally in the distal convoluted tubules and the collecting tubules of the cortex. The presence of these casts, alone or with occasional invading monocytes, was not associated with evidence of surrounding edema, but when the casts were heavily infiltrated with cells and when the epithelium was disrupted, there were changes in the surrounding connective tissue consisting of edema and infiltration with cells identified as polymorphonuclear leukocytes, monocytes, lymphocytes and occasional eosinophils in varying proportions. The cell type bore no relation to the duration of the jaundice. Mitotic figures were rare or absent in spite of such evidence of tubular degeneration. These changes had not resulted, in any instance, in the formation of cicatrices.

In 14 cases there was phagocytosis of pigmented cast material by epithelial cells. This always occurred in the distal convoluted and collecting tubules. Although these lesions were encountered more often in cases 3, 5, 10 and 18, they were so few that they were found only after prolonged search.

In only 2 cases were there numerous large pigmented casts in the collecting tubules deep in the pyramids, and these tubules and casts were free from cellular infiltration.

Glomerular lesions were found in only 7 cases. Four of these showed rare, isolated, partly hyalinized bloodless loops without epithelial proliferation; one showed slight increase in the total number of nuclei (case 18), and one showed a rare hyalinized loop and an increase in nuclei (case 8). In both of the cases the tuft capillaries uniformly contained little or no blood.

The blood vessels contained no thrombi; the vessel walls were normal.

The severity of the renal changes was not in any way related to the various changes in the livers.

COMMENT

That morphologic changes occur in kidneys in the course of severe jaundice has long been accepted. The details of these changes have not been so generally agreed on. Werner,⁴ using rabbits and producing jaundice by subcutaneous injections of ox bile, found degeneration of the tubules. This degeneration consisted of pigmentation, vacuolation, cloudy swelling and desquamation of the tubular epithelium, principally that of the convoluted tubules. The glomeruli were normal. Romeo,⁵ using 7 dogs in which the common bile ducts had been ligated, found the principal changes in the glomeruli. These consisted of glomerular engorgement, subsequent atrophy and finally sclerosis of glomeruli. The tubular degeneration was similar to that described by Werner but was considered of secondary importance. These dogs had albuminuria, changing to oliguria about the thirty-fifth day of jaundice. In 13 adults with so-called bile nephrosis (Wilbur⁶) there was no oliguria, but albuminuria was a constant finding. The kidneys from these patients showed tubular degeneration, but the glomeruli were histologically normal. Despite these differences the authors agreed that the epithelial cells of the tubules and the casts were stained with bile, and the first two authors felt that the degenerative changes were caused by bile.

Horrall and Carlson⁷ showed that it is the bile salts and not the bile pigments that produce functional renal damage in experimental animals. No tests were made for bile salts in the present series. Consequently, it can only be assumed that bile salts passed through the kidneys. If so, they did not produce hematuria or oliguria. The non-protein nitrogen of the blood was normal in 1 case (15) and, although the blood was not examined chemically in 17 cases, there were no signs to lead the clinicians to suspect uremia. In only 6 of these cases was albuminuria shown, and in these a trace of albumin was found only in the last specimens examined before death. Apparently, the edema in

4. Werner, R.: *Arch. f. exper. Path. u. Pharmacol.* **24**:31, 1887.

5. Romeo, M.: *Ann. ital. di chir.* **8**:1376, 1929.

6. Wilbur, D. L.: *Arch. Path.* **18**:157, 1934.

7. Horrall, O. H., and Carlson, A. J.: *Am. J. Physiol.* **85**:591, 1928.

cases 4, 14 and 16 was not due to the demonstrable lesions of the kidneys, because the tubular lesions in these cases with edema were the slightest in their respective groups. Thus, it seems permissible to consider that these renal lesions occurred without clinical evidence of serious impairment of renal function.

Boyce and McFetridge⁸ suggested that renal failure occurs not during jaundice but after sudden release of the bile duct obstruction. In this connection additional cases in which congenital atresia of the bile ducts was relieved by operation are of interest. Release of the obstruction was carried out in 6 cases. In none of these was anuria or any evidence of renal impairment observed before or after operation. Gross⁹ recently followed up 5 of these 6 cases from five to thirteen years and found no clinical evidence of renal disease. However, no kidneys from infants in whom such obstruction had been relieved were available for study.

Despite the lack of clinical evidence of oliguria in the cases coming to autopsy, there is a strong similarity between the lesions in these kidneys and those described in transfusion reaction with anuria. In the latter condition the anuria has been explained by considering that the casts in the collecting tubules produced mechanical blockage.¹⁰ It is true that in many cases of transfusion reaction the kidneys show much more blockage of the tubules by casts than do the more severely involved ones in the present series (cases 1, 2 and 6), but in some instances the kidneys show much less blockage.

It is beyond the scope of this paper to discuss the renal lesions of mercury bichloride poisoning. Occasionally, however, the kidneys in transfusion reaction and in the so-called hepatorenal syndrome with anuria resemble those in mercury bichloride poisoning.¹¹ Some degree of resemblance might be expected because of the wide range of microscopic tubular lesions in mercury bichloride poisoning. Such kidneys may show no lesions.¹² They may also show severe cloudy swelling and desquamation of the epithelium of the convoluted tubules or deep acidophilic staining of the granular dead cells;¹³ or later, during regeneration, only low deeply basophilic cells of the convoluted tubules with mitotic figures.¹⁴ Heineke^{14b} concluded that neither the degenerative

8. Boyce, F. F., and McFetridge, E. M.: *Arch. Surg.* **32**:1080, 1936.

9. Gross, R. E.: Personal communication to the author.

10. DeGowin, E. L.; Warner, E. D., and Randall, W. L.: *Arch. Int. Med.* **61**:609, 1938. Bell.²

11. DeGowin, E. L., and Baldrige, C. W.: *Am. J. M. Sc.* **188**:555, 1934. Lichtman, S. S., and Sohval, A. R.: *Am. J. Digest. Dis. & Nutrition* **4**:26, 1938 [Case 1].

12. Hunter, W. C.: *Ann. Int. Med.* **2**:796, 1928.

13. MacNider, W. de B.: *J. Exper. Med.* **27**:519, 1918.

14. (a) Shapiro, P. F.: *J. Lab. & Clin. Med.* **15**:961, 1929. (b) Heineke, A.: *Beitr. z. path. Anat. u. z. allg. Path.* **45**:197, 1909.

changes of the epithelium nor the infiltration and edema of the stroma were peculiar to mercury bichloride poisoning. He felt, however, that certain sequences of inclusion or of phagocytosis of the necrotic material by the epithelial cells were of diagnostic importance. Although none of these jaundiced kidneys had mitotic figures in the tubular epithelium and none had low basophilic epithelium, there were 14 in which phagocytosis of cast material by epithelial cells was taking place. Yet there was no history of exposure to heavy metal in the cases, and no calcium was shown in the kidneys.

Finally, the lack of relationship to pyelitis should be stressed. In the single case (12) in which pyelitis was obvious clinically and post mortem the kidneys were most nearly normal, showing only rare casts and no edema or cellular infiltration; while in the case in which the lesions of the kidneys were most severe (case 2) no infiltration of the pelves was found and bacterial stains failed to demonstrate organisms. In addition, the large number of cases in which there was no clinical or postmortem evidence of pyelitis makes it seem unlikely that this infection is in any way related to the tubular lesions in these infants.

The changes in the proximal convoluted tubules were all of the type produced by Jackson¹⁵ in rats by keeping them on a high protein diet.

An understanding of the real cause and pathogenesis of these lesions must await further experiments on animals. Possibly the bile salts alone may produce such changes, or possibly they are produced by some substance, as yet unidentified, which is secreted normally by the liver. Whatever the substance is, it does not produce clinically important renal damage during jaundice or after relief of jaundice in infants with congenital atresia of the bile ducts.

SUMMARY

In infants with long-sustained uncomplicated jaundice, due to congenital atresia of the bile ducts, renal lesions occur without producing oliguria. These lesions consist of focal exudative changes, obstruction of tubules by casts and phagocytosis of cast material by epithelial cells. These lesions are duplicated in the kidneys from patients with transfusion reaction and the hepatorenal syndrome, to be reported on subsequently.

15. Jackson, H.: *Am. J. Path.* **3**:285, 1927.

HISTOLOGIC SEQUENCES IN THE MENINGIOMA, WITH A CONSIDERATION OF THE NATURE OF HYPEROSTOSIS CRANII

ORVILLE T. BAILEY, M.D.

BOSTON

The list of publications dealing with the nature of the meningioma has become so long and so distinguished that the addition of another paper taking up the question requires considerable temerity. Yet these publications have expressed such diverse opinions and have brought forward such apparently contradictory data that an understanding of the tumor must await a new approach and a synthesis of these various studies. The possibilities of growth sequences and of participation in the meningioma of non-neoplastic tissues have especially been neglected.

The term "meningioma" is used in this paper to designate that common tumor of the meninges which is composed of elongate cells, often arranged in whorls or palisades, and which in certain locations is associated with an irregular proliferation of the overlying bone. The imposing group of terms which have been applied at one time or another to this familiar neoplasm—"dural endothelioma," "arachnoid fibroblastoma" and many others—stands as witness to the changing conceptions of the tumor and of the meninges from which it originates. These neoplasms are regarded in this paper as a distinct type; melanoma, lipoma and other tumors of the meninges, classified by some writers as varieties of meningioma, are here excluded from it.

Studies dealing with meningioma have sought, for the most part, to identify, in the tangle of intercellular fibrils, whorls and laminated concretions, cells which have unmistakable resemblances to cells known in normal tissues¹ or to match the appearance of the tumor in an average field with some stage in the embryonic development of the meninges.²

From the Departments of Pathology of the Harvard Medical School and of the Peter Bent Brigham Hospital, Boston, and the Society of Fellows, Harvard University, Cambridge, Mass.

1. (a) Mallory, F. B.: *J. M. Research* **41**:349, 1920. (b) Oberling, C.: *Bull. Assoc. franç. p. l'étude du cancer* **11**:365, 1922. (c) Penfield, W.: *Surg., Gynec. & Obst.* **45**:178, 1927.

2. (a) Bailey, P., and Bucy, P. C.: *Am. J. Cancer* **15**:15, 1931. (b) Globus, J. H.: *Arch. Neurol. & Psychiat.* **38**:667, 1937. (c) Cushing, H., and Eisenhardt, L.: *Meningiomas: Their Classification, Regional Behavior, Life History, and Surgical End Results*, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

This investigation of the nature of the meningioma has been undertaken along somewhat different lines. It begins with the premise, now agreed on by most of those who have studied neoplasms of this type, that all tumors properly falling into the category of the meningioma have a common origin in the type of cell which forms the superficial layer of the arachnoid membrane, whether found there or in the pacchionian granulations. By means of the known properties of the arachnoid cell, this study seeks to identify a series of histologic sequences which may account for the morphologic character of the meningioma, both in the form most frequently recognized and in the variants which appear in any large series of these tumors.

MATERIALS AND METHODS

At the Peter Bent Brigham Hospital there were available for study 27 examples of meningioma, encountered between Sept. 1, 1932 and Sept. 1, 1939 (after excluding 2 examples in Dr. Cushing's service during that period). Material from an additional patient came from the Boston Children's Hospital. This was the only meningioma in the extensive series of brain tumors of childhood collected at that hospital, a fact which emphasizes the rarity of this neoplasm in early life. While no material from the great series of Dr. Harvey Cushing has been used, impressions from his records and material in the files of this hospital are unavoidable. In 15 of the 28 cases of the series, blocks of tumor tissue 1 to 2 mm. thick were fixed within ten minutes after surgical removal of the growth and were placed in large quantities of Zenker's fluid. Other blocks of tumor tissue of similar character were fixed in a 4 per cent solution of formaldehyde. It was on these 15 perfectly preserved tumors that chief reliance was placed for study of cellular details. All the other tumors were well fixed, any material not showing good histologic detail being discarded. The staining methods used routinely were those of Mallory: eosin-methylene blue, phosphotungstic acid-hematoxylin and aniline blue-acid fuchsin-phosphomolybdic acid. Many of the tumors were also stained with Masson's trichrome stain, Foot's modification of the Bielschowsky-Maresch method for reticulum, and Weigert's resorcin-fuchsin stain for elastic tissue. The material from the hyperostoses was embedded in pyroxylin (celloidin) after decalcification with nitric acid, and the sections were stained with hematoxylin and eosin.

THE RELATION OF THE ARACHNOID CELL TO THE MENINGIOMA

As stated earlier, this study has regarded as established the view that the meningioma takes origin in cells of the type of those which form the outer layer of the arachnoid membrane whether they are found in that location or in the arachnoid villi. The evidence on this point is derived from several sources, among the more important of which are: the similarity in morphologic details between the arachnoid cells and the cells of the meningioma,³ the coincidence between the location of arachnoid villi and the favored sites of origin of the meningioma,⁴ and the

3. Schmidt, M. B.: *Virchows Arch. f. path. Anat.* **170**:429, 1902.

4. Cushing, H.: *Brain* **45**:282, 1922.

presence of whorl patterning and laminated calcareous concretions both in arachnoid cell clusters and in the meningioma.⁵ Further important evidence lies in the fact that there are some tumors of this type which arise in sites remote from dural contact and others, originating nearer the dura, which have no demonstrable attachment to this structure.⁶ The observations on the present series are in accord with this view. Yet it has been shown^{1a,c} that certain of the cells in the meningioma are fibroblasts. Are the arachnoid cells fibroblasts, or is it possible that more than one tissue participates in the development of the meningioma? Is more than one element neoplastic? If the meningioma consists of more than one type of tissue, one should be able to demonstrate in a series of these tumors histologic sequences leading to the formation of whorls, laminated concretions and other features which are found mentioned in textbook descriptions of this tumor. The point of departure, then, in unraveling these sequences is a study of the behavior of the tissue elements certainly present, the arachnoid cell, under normal conditions and in various pathologic states.

THE NORMAL ARACHNOID CELL

The embryologic origin of the arachnoid cell, in spite of much effort, must be considered still in question. From the earlier view that it is mesodermal in origin,⁷ there has been a shift to the position that it is derived from the neural crest, the change of view coming about largely as a result of the experimental studies of Harvey and Burr.⁸ Flexner,⁹ however, interpreted similar experiments as indicating the participation of both entodermal and ectodermal mesenchyme in the formation of arachnoid cells. This paper has been answered and the experiments considerably extended by Harvey, Burr and van Campenhout;¹⁰ these workers' findings have been further confirmed by Raven's studies on Triton.¹¹ If one wishes to hold rigidly to the theory of specificity of germ layers, the evidence now at hand seems to indicate that the arachnoid cells are derived from the neural crest. Even if that theory is not entirely correct, these investigations indicate that the arachnoid cells develop in tissue largely of neural crest origin. It makes little difference in the present study of the meningioma from what germ layer the arachnoid cell is ultimately found to be derived. The point which is essential here is

5. Weed, L. H.: Bull. Johns Hopkins Hosp. **31**:343, 1920.

6. Cushing and Eisenhardt,^{2c} p. 133.

7. Weed, L. H.: Contrib. Embryol. (no. 14), publication 225, 1917, p. 3.

8. Harvey, S. C., and Burr, H. S.: Arch. Neurol & Psychiat. **15**:545, 1926.

9. Flexner, L. B.: Contrib. Embryol. (nos. 110-117) **20**:31, 1929.

10. Harvey, S. C.; Burr, H. S., and van Campenhout, E.: Arch. Neurol. & Psychiat. **29**:683, 1933.

11. Raven, C. P.: Arch. f. Entwcklungsmechn. d. Organ. **134**:122, 1936.

whether the arachnoid cell is a fully differentiated cell and whether this differentiation is maintained in the meningioma or whether it is an undifferentiated fibroblast, as Mallory^{1a} and his followers have suggested.

THE MENINGIOMA A TUMOR OF SPECIALIZED NEOPLASTIC
CELLS AND STROMA

In its normal position the arachnoid cell is thin and flat with deeply staining homogeneous cytoplasm and a discrete, centrally placed nucleus. The morphologic characteristics of the arachnoid cells persist in most meningiomas. Careful study of well fixed material, however, brings out certain variants. There are a few extremely rapidly growing meningiomas in which there is marked variation in the size and shape of the cells, and even giant cell formation.¹² These are characteristics of cellular growth well known to be associated with rapid growth in tumors, whatever their site of origin. Such variations are to be expected in a series of tumors of any type and do not have direct bearing on the nature of the lesion in question. For the present purposes it is much more significant that among the cells which have an unmistakable resemblance to normal arachnoid cells there are fibroblasts with well developed fibroglia fibrils, sometimes numerous, sometimes rare but seldom absent. Mallory^{1a} demonstrated fibroglia fibrils in large numbers in a particularly well fixed and stained meningioma and later found them in several other meningiomas. He therefore proposed that the cells of the meningioma are of fibroblastic origin, the fibroblasts with fibroglia fibrils being the only completely differentiated tumor cells. Penfield^{1c} in a study of his series of meningiomas demonstrated at least a few fibroglia fibrils in each of the specimens. Others have followed the lead of Mallory and Penfield in regarding the tumor as an arachnoid fibroblastoma. Yet still other investigators have held that all the cells of these tumors can by no means be regarded as fibroblasts in any stage of differentiation.

In the present series there were 15 tumors which were treated technically with the greatest care, thin blocks being placed in large amounts of Zenker's fluid ten minutes or less after surgical removal of the tumor. In 10 of the tumors there were fibroglia fibrils; in 5 there were none demonstrable after long search. Furthermore, in the tumors containing few or no cells with fibroglia fibrils the neoplastic cells did not resemble any stage of development of the fibroblast as it is seen elsewhere in the body or under other conditions. The neoplastic cells did, however, present a distinct similarity to normal arachnoid cells. In some instances the tumor cells were larger and more rounded than these cells as seen in the leptomeninges and arachnoid villi, but transitions from them to the

12. See, for example, the case of Dorothy May Russell, described by Cushing and Eisenhardt,^{2c} p. 692.

familiar flattened form of the arachnoid cells could be located after some search. The neoplastic cells in these tumors resembled, in morphologic aspect and especially in method of growth, epithelial cells rather than fibroblasts. When the tumors containing numerous cells with fibroglia fibrils were examined many cells were readily found which resembled the arachnoid cells. There seemed to be no intermediate stages between them and the fibroblasts associated with the fibroglia fibrils.

An understanding of the meningioma involves an explanation of the presence of arachnoid cells and fibroblasts in the same tumors. To Mallory ^{1a} and others, who have followed his ideas in this regard, the fibroblasts are the mature tumor cells, those resembling the arachnoid cells the immature, incompletely differentiated ones. If all the cells of the meningioma are to be regarded as fibroblasts in varying degrees of differentiation, the arachnoid cells under normal conditions must be undifferentiated, for the resemblance of the two is unescapable. Both Mallory ^{1a} and Penfield ^{1c} have been willing to go that far. It seems remarkable that under normal conditions a position so important as the surface of the arachnoid should be covered by cells of immature character. Mallory ^{1a} pushed the argument even farther by suggesting that the power of growth in the arachnoid is limited to the surface layer of incompletely differentiated cells. Study of many sections of all parts of the leptomeninges in this laboratory have failed to show any evidence of transition cells between those of the superficial arachnoid layer and the mature fibroblasts beneath them. The numerous mitotic figures found in such germinative layers as that of the skin are not present in the cells of the superficial layer of the arachnoid. While the arachnoid cells participate in various forms of physiologic activity,¹³ there is no evidence that they assume the form of fibroblasts under any of the widely different conditions in which they have been tested.

It is certain that many meningiomas contain typical adult fibroblasts. If these cells are not the fruits of a maturation process in the tumor cells, how is their presence to be explained? The scheme set up in this paper proposes that the surface layer of the arachnoid is covered by cells which are highly specialized and which are especially adapted as covering cells. This would imply that the underlying connective tissue of the normal arachnoid acts as a supporting and nutritive tissue for the covering layer. If this is the normal relationship between the layer of arachnoid cells and the tissue beneath them, the meningioma would be expected to consist of arachnoid cells in association with a stroma stimulated by the action of these cells on connective tissue in the region of the tumor.

References are made to the stroma of the meningioma in many papers dealing with this type of tumor (Mallory; ^{1a} Penfield,^{1c} and others). The

13. (a) Weed.⁵ (b) Essick, C. R.: *Contrib. Embryol.* (no. 42) 9:377, 1920.

writers of these papers have, for the most part, regarded the stroma of the meningioma as comparable to that of the fibroblastoma, i. e., that it is made up only of blood vessels and the accompanying connective tissue while the remainder of the intercellular material is either produced by the tumor cells or consists of bits of tissue invaded but not destroyed by the neoplasm. Now this view of the stroma of the meningioma presents certain important difficulties, especially when a large series of these tumors is considered. On analysis the arrangement of the tumor cells does not have the complete irregularity of the connective tissue seen in the fibroblastoma; on the contrary, there are masses of cells set off from one another by fibrous tissue which does not appear to be laid down by the neoplastic cells. On further study of the cell masses they are found to be composed of groups of cells of arachnoid cell type with fibroblasts and connective tissue fibers at their periphery. In some areas the fibroblasts replace the tumor cells, a process of partial sclerosis such as is seen in many tumors of neoplastic cells and stroma (for instance, carcinoma of the breast). If the meningioma were a fibroblastoma, the most slowly growing one should be that composed of cells most like adult fibroblasts. The study of the carefully followed series published by Cushing and Eisenhardt ^{2c} has shown that such is not the case.

It is suggested, then, that the meningioma is a tumor of specialized cells embedded in and dependent on a stroma which they themselves have stimulated. If this represents the actual nature of the meningioma, it should be possible to trace certain sequences of growth which would lead to the characteristic morphologic aspects of the meningioma. The problem may be divided into two parts: first, the character of the specialization of the arachnoid cell and, second, the evidence found under special conditions for the presence of a stroma in essential relationship to the tumor cells. It is proposed to begin the discussion by a consideration of the first of these divisions.

GROWTH SEQUENCES IN THE MENINGIOMA DEPENDENT ON THE SPECIALIZATION OF THE TYPE CELL

There is much evidence that the cells of the outer layer of the arachnoid are, both in morphologic nature and in function, different from the cells facing them across the subdural space on the inner surface of the dura. Mallory ^{1a} stated, "There is no dural endothelium," and brought forward important morphologic evidence bearing on this point. This statement received additional support from certain experimental studies. It was shown ¹⁴ that defects made operatively in the dura without injury to the underlying arachnoid heal without formation of adhesions between the two layers, but that when the pia-arachnoid is injured and the over-

14. Savad, W. Y., and Harvey, S. C.: *Ann. Surg.* **77**:129, 1923.

lying dura left intact, adhesions between the two layers develop.¹⁵ This difference in repair on the part of the two membranes indicates that the arachnoid cells differ from those lining the inner surface of the dura. The former act as a specialized limiting membrane, which is not readily replaced once it is traumatized, while the latter participate in the repair process as does collagenous connective tissue wherever found.



Fig. 1.—A whorl about a collagen fiber. The section has been stained by Mallory's aniline blue method. The only material taking the aniline blue stain is the central fiber, shown in cross section (and intense black in the drawing). Cells resembling those of the superficial layer of the arachnoid are wound around it in a compact nest. Farther out are elongated cells, some associated with fibroglia fibrils; these cells enclose the group of tumor cells and belong to the stroma of the neoplasm. This is a camera lucida drawing at a magnification of 1,300 diameters.

Further evidence on this point was brought forward by Leary and Edwards,¹⁶ who showed that scrapings from the surface of the arach-

15. Lear, M., and Harvey, S. C.: *Ann. Surg.* **80**:536, 1924.

16. Leary, T., and Edwards, E. A.: *Arch. Neurol. & Psychiat.* **29**:691, 1933.

noid (composed almost entirely of arachnoid cells) resemble those from such serous cavities as the pericardium, while scrapings prepared by a similar technic from the inner surface of the dura are entirely different in appearance, resembling tissue cultures of fibroblasts. There is evidence of several types, therefore, that the cells facing each other on opposite sides of the subdural space are different and that the outer layer of the arachnoid is a specialized covering layer, while the inner portion of the dura is composed of fibroblasts and fibrils formed by them. The arachnoid cells invest the brain and spinal cord on their exterior with a delicate sheath which allows a certain independence in motion from the more rigid outer covering.

If this conception of the arachnoid cell is significant, it might be found behaving as a covering cell in the tumors which arise from it. A frequent but by no means constant feature of the histologic picture of the meningioma is the whorl. In these cell groups the tumor cells are closely wrapped together in a concentric manner. There is at times one or a few collagen fibers at the center of a whorl (fig. 1) or less commonly an elastic tissue fiber. At other times the whorl is built about a small blood vessel (fig. 2). In many instances the tumor cells are tightly wrapped about one another, without a nidus being present. Such behavior is interpreted as a continuation of the tendency of the arachnoid cells to act as covering cells. The collagen fibers are covered; so are the small blood vessels; even tumor cells are covered by other tumor cells, building up the whorl in layer after layer.

In the smallest whorls (and the youngest so far as age can be judged from morphologic appearances alone) the cells most closely applied to the central nidus are typical arachnoid cells while more peripherally there may be cells resembling fibroblasts which act as supporting tissue for the cell cluster (figs. 1 and 2). The fibroblasts form columns, which are not so tightly coiled about the cluster of cells but pursue a straighter course. In such whorls, collagen is lacking or is confined to the region of cells which morphologically are clearly fibroblastic. Mallory^{1a} classified the whorls of the meningioma as cellular whorls, such as those described in the foregoing paragraph, and fibrous whorls, in which there are concentric layers of collagen fibers with or without elastic fibers. When the fibrous whorls become calcified, the concentrically laminated concretions called "psammoma bodies" are formed. Mallory^{1a} pointed out that the amount of connective tissue in the fibrous whorls is greatly in excess of that in other parts of the tumor. He said, "They are evidently due to fibroblasts which have been included in cellular whorls of the tumor and have been stimulated to unusual activity." From the context it is possible, but not certain, that Mallory referred to fibroblasts of the dura and not to cells of the tumor (which he regarded as fibroblasts

also). If the tumor cells are fibroblasts in one degree of differentiation or another, it is difficult to see how inclusion of a fibroblast in a mass of similar cells would lead to greater production of collagen. If the tumor cells are regarded as specialized arachnoid cells stimulating a stroma from surrounding tissue for their support and nutrition, the inclusion of fibroblasts in a whorl would have the effect of subjecting them to the maxi-

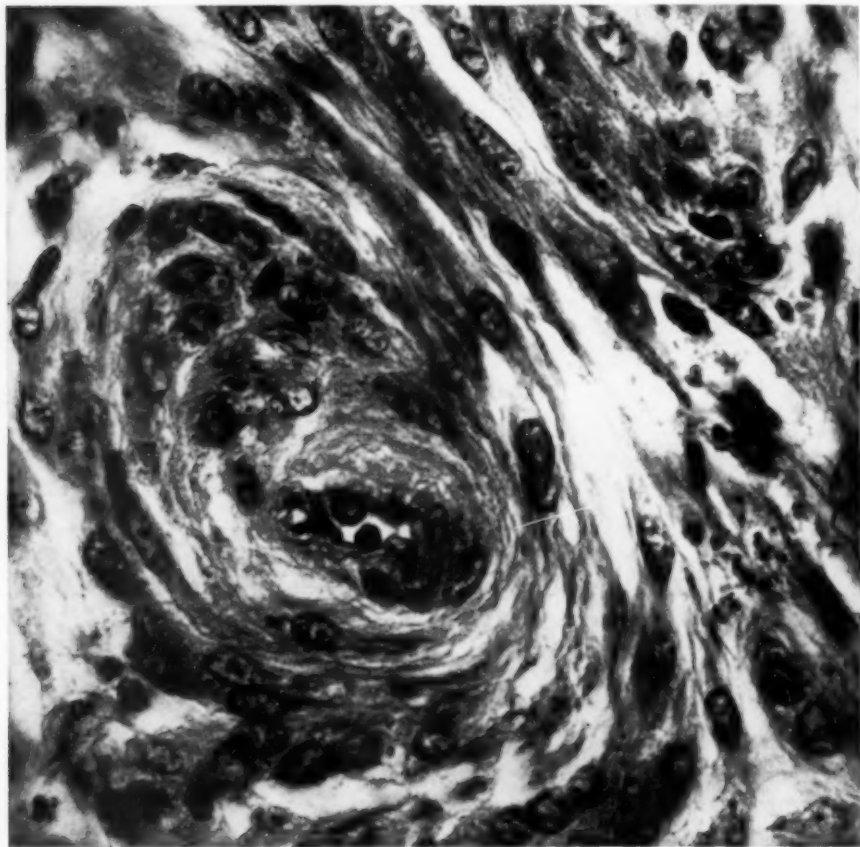


Fig. 2.—A whorl about a capillary. The tumor cells, of arachnoid cell type, are closely applied to the vessel to give rise to a cellular cluster as a result of the tendency of these cells to cover structures which they reach in growth. Farther from the vessel are elongate cells, some of which are associated with fibroglia fibrils. These are cells of the tumor stroma and not primary neoplastic cells. Mallory's phosphotungstic acid-hematoxylin; magnification, 900 diameters.

imum desmoplastic effect of the tumor cells. While in the very smallest whorls the fibroblasts are segregated peripherally, it is in keeping with the behavior of stroma in other tumors for the fibroblasts to extend into

the groups of neoplastic cells as growth continues. It is common to find evidence of degeneration of tumor cells in whorls, both cellular and fibrous. This introduces the possibility that the fibrous whorl may at times result from condensation of the stroma of a larger whorl in which the neoplastic cells have partially or wholly degenerated. The fibrous whorl may be the end result of several series of processes. It is usually impossible to detect the starting point and the route traveled if only the destination is known.

The concentrically laminated concretions (psammoma bodies) are a feature of the meningioma which has received undue prominence from the fact that the tumor was once named from their presence and because they are so striking in that special variety of meningiomatous structure which is described and illustrated in textbooks. The psammoma is really a further step beyond the fibrous whorl in the way of cellular degeneration and replacement. In the fully developed psammoma there are concentric layers of calcified material. Pathologic calcium deposits depend on several factors, one of which is the presence of a matrix of dead tissue. Such a matrix could result from the necrosis of tumor cells. These considerations tend to indicate that the so-called fibrous whorls and psammoma bodies result from degeneration of tumor cells with persistence and apparent overgrowth of stroma rather than from any special propensity of the tumor cells themselves.

While the whorl is a striking feature of many meningiomas, it is only suggested in some of these tumors, and in a considerable percentage it is entirely lacking. Occasionally, it is possible to find noncircular structures which are covered with arachnoid cells or cell groups about circular structures cut in planes other than a diameter. The result is not a symmetric whorl but a less regular mass of cells which follows the outline of the structure about which the cluster is built up. There is a tendency in some meningiomas toward palisading of cells. The stated frequency of this histologic feature varies a good deal in various descriptions, depending on how conspicuous the arrangement must be before an author applies the term. From the study of this series and a survey of the literature it appears that the alinement of a few nuclei or a less regular parallelism of several is rather frequent but that definite and striking palisading as seen in neurofibromas is exceptional. The palisade-like grouping has been shown by Penfield^{1c} to be determined by the arrangement of the connective tissue fibers among the tumor cells. When the connective tissue fibers are distributed in a somewhat different way, clusters and eddies of tumor cells result. It seems that these various groupings of neoplastic cells are better explained by the covering tendency of arachnoid cells than by wholly mechanical compression. It should be recalled that the leiomyoma at times shows similar cell grouping.^{1a} It is difficult to fit any single explanation to both these varieties of tumor, and

it may be that what is called palisading can result from various factors under different conditions. Palisading is not the result of pure chance, since many tumors of flattened or spindle-shaped cells show it rarely if



Fig. 3.—A meningioma in which the tumor cells and the stroma are especially sharply demarcated. The tumor cells form compact masses and are deeply stained; these groups are separated from one another by a distinct fibrous tissue stroma. Eosin-methylene blue stain; magnification, 165 diameters. See also figure 4.

at all (osteogenic sarcoma, spindle cell carcinoma of the lung). The matter is further complicated by the fact that certain cellular meningiomas bear a resemblance to neurofibromas, tumors in which palisading is much more striking than it is in meningiomas. This possible relationship is discussed later.

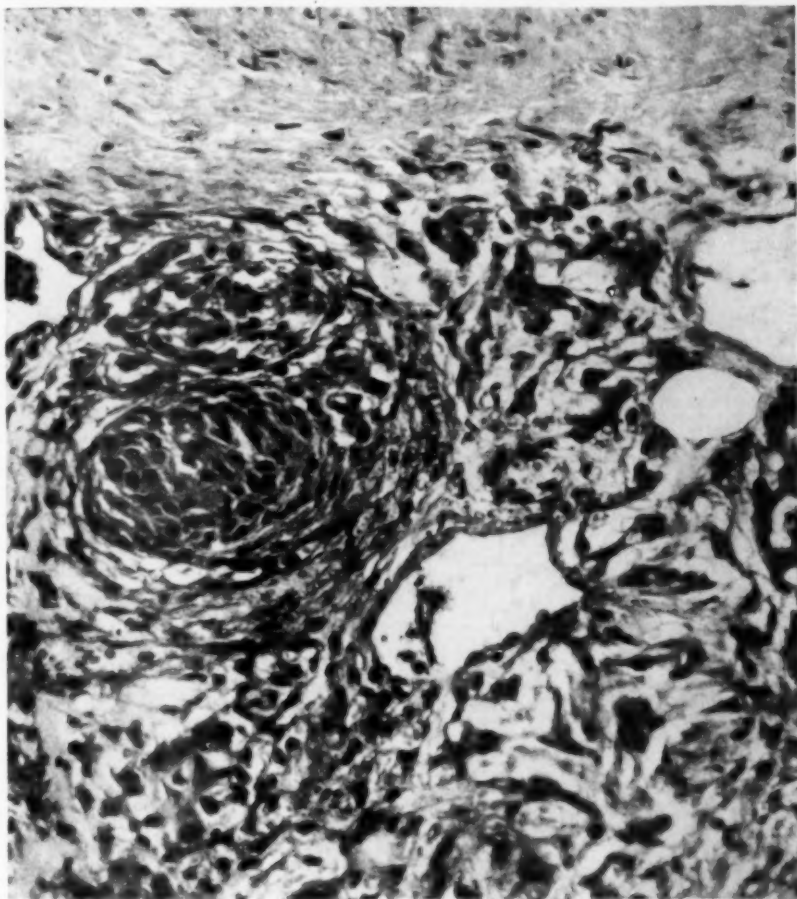


Fig. 4.—Another area from the same meningioma as that of figure 3. The dura is shown at the top. The prevailing histologic appearance of the tumor is shown at the bottom and right of the figure. At the left of the center there is a small whorl. The occurrence of small whorls in meningiomas otherwise so definitely divided into neoplastic cells and fibrous stroma emphasizes that the whorl is the result of growth sequences depending on the juxtaposition of these two distinct varieties of cells. Eosin-methylene blue stain; magnification, 165 diameters.

Occasionally there is a meningioma which is composed in part or even entirely of small groups of tumor cells in a clearly differentiated stroma (figs. 3, 4 and 5). The areas in which this variety of histologic

structure is present are the ones in which the "epithelial" character of growth in the meningioma is most clearly indicated. Here the tumor cells exhibit no detectable signs of functional activity. In other neoplasms

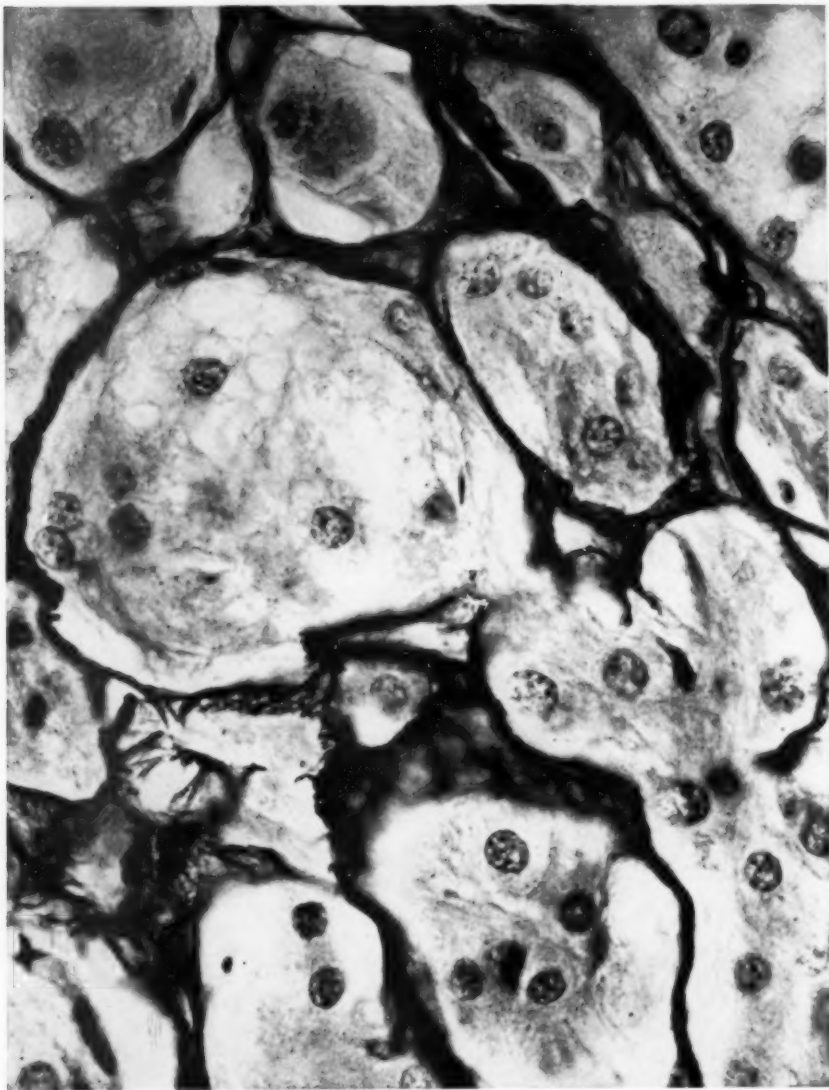


Fig. 5.—Groups of neoplastic cells separated from one another by minute connective tissue fibers (reticulum), which are rendered intensely black by silver impregnation. This is a photomicrograph, at a magnification of 900 diameters, of a meningioma stained by Foot's method for reticulum and counterstained with Van Gieson's stain.

in which the cells give evidence of functional activity there are areas, usually numerous, in which it is suppressed. It is interesting that in the meningioma the areas in which there is no evidence of a tendency on the part of the tumor cells to cover something should be those in which the stroma and neoplastic cells are most clearly separated. When the tumor cells do not wind around some structure which they have included from preexisting tissue or which they have stimulated for a stroma, the relation of the tumor cells to their supporting elements becomes much clearer. This comes about because of the absence of the successive growth sequences which have done so much to obscure the nature of the meningioma. Cushing and Eisenhardt²⁰ showed that the average prognosis of meningiomas with the conventional whorls and psammoma bodies is about the same as that of meningiomas without these features. On the other hand, in a small group of very rapidly growing tumors in their series (designated by them type VI, variant 1) whorls are entirely absent. Functional activity is least apt to appear in the most rapidly growing tumors of any type, but the more slowly growing ones may or may not show it. There are evidently factors other than time which are important for the development of functional activity in tumors. This in no way affects the fact that such evidences of functional behavior on the part of tumor cells are of prime importance in unraveling their origin and method of growth.

The meningioma has been discussed as a tumor of functional cells with a fibroblastic stroma. At times tumors with stroma originating in various organs stimulate additional stroma from cells which produce something besides collagen fibers and blood vessels. Then, if the tumor is not so rapidly growing that it destroys its own stroma in further progression or kills the patient before the stroma has an opportunity to lay down its special products, these particular varieties of intercellular material are of assistance in studying the source of the stroma and its behavior when incorporated in the tumor. The two most important of these stroma products in the meningioma are elastic tissue and bone.

ELASTIC TISSUE IN THE STROMA OF THE MENINGIOMA

Elastic tissue is extensively distributed through some meningiomas, but in a greater number of them it is absent except in the walls of the larger blood vessels and in the dura adjacent to the tumor. In the present series there were only 2 tumors with considerable amounts of elastic tissue elsewhere in the neoplasm. Its distribution in these tumors corresponded in general to that described by Mallory.¹⁰ The elastic tissue of portions of dura through which the tumors had extended was increased as though stimulated by the tumors, as in Mallory's cases. Isolated elastic tissue fibers were scattered irregularly among the nearby tumor cells, single fibers at times spreading outward from the bundle

at the edge of the dura. The elastic fibers of the dura and large blood vessels stained deeply and uniformly by Weigert's resorcin-fuchsin method. Within the meningiomas the elastic tissue of the adventitia of larger arteries was increased and in places extended among the tumor cells so as to become an integral part of the stroma. In one of the tumors there were fibrils which were stained in a pale, irregular manner by Weigert's technic. These paler fibers were considered evidence of new formation of elastic tissue. The presence of such fibers in meningiomas has been pointed out by Mallory,¹⁸ Van Wagenen¹⁷ and others. While elastic tissue is not frequently found as a part of the stroma in tumors, certain special tumors stimulate its growth to form part of their supporting networks—for example, the argentaffinoma. A study has been made of the derivation of elastic tissue in the stroma of the argentaffinoma.¹⁸ Figure 6 of the paper in which the results were presented¹⁸ shows that the source of some of the elastic tissue is the adventitia of large arteries. Although not shown in the illustration, some of the elastic tissue fibers at a distance from the artery took the pale, irregular stain with Weigert's resorcin-fuchsin that characterizes newly formed elastic tissue. These young elastic fibrils could not have been formed by the argentaffin cells; they must therefore be regarded as a part of the stroma stimulated by the tumor cells. This behavior of the elastic tissue at the adventitia of large blood vessels is duplicated in the single example in the present series showing definite evidence of new elastic fibril formation. The conclusions reached from the study of this material are that the elastic tissue in most instances of its occurrence in meningioma is that of the dura and adventitia of large arteries which has come to be included by the advancing tumor and has spread out among its cells, that under certain undetermined conditions the meningioma cells (of arachnoid cell character) may stimulate the formation of new elastic tissue fibers from either of the two sites just as the tumor cells stimulate the production of new collagenous tissue and blood vessels and that such growth of new elastic tissue fibers may be over a distance that cannot be completely followed in a histologic section.

THE NATURE OF HYPEROSTOSIS CRANII

A question regarding meningioma over which there has been much discussion is that of the nature of the hyperostosis associated with it in many instances (9 instances among the 28 of the present series). Can the hyperostosis be explained by regarding the meningioma as a tumor with a stroma? The evidence at hand indicates not only that this can be done but also that the strongest confirmation for the theory comes from a study of the hyperostoses.

17. Van Wagenen, W. P.: *Arch. Surg.* **18**:1621, 1929.

18. Bailey, O. T.: *Arch. Path.* **18**:843, 1934.

The past investigations of hyperostosis cranii have indicated that the hyperostosis is secondary to the meningeal tumor and dependent in some way on it for development.¹⁹ Other views of the relationship between the meningeal neoplasm and the overgrowth of the adjacent bone are of historical interest only.²⁰

In studying a series of meningiomas associated with hyperostoses attention is directed to the peculiar fact that both the soft and the bony parts are composed of a single type of tumor cell but that in one part there is extensive bone formation and in the other there is none.²⁰ On further study the bone is found to be continuous with adjacent normal bone except in a few unusual instances in which it is different in nature and which are discussed separately in the next section of this paper. Some meningiomas, although lying against bone, do not cause any change in the osseous tissue; a few others invade the bone and destroy it without associated production of new bone; others are associated with growth of bone outward from the skull, others with growth inward, and still others with proliferation of osseous tissue in both directions.

The growth of bone is progressive and the hyperostosis may become very great. The grotesque growths occurring before the development of modern surgery, illustrations of which were collected from the literature by Cushing and Eisenhardt,²⁰ are ample evidence in point. Microscopic examination of the bone in an area of hyperostosis shows that the mosaic pattern is entirely altered (fig. 6). It is irregular and presents wide variations in different parts of a single lesion. Cushing¹⁹ pointed out that a hyperostosis may occur in the absence of increased intracranial pressure. These facts are conclusive evidence that the meningioma cells do not appear in the interstices of the hyperostosis because they have been squeezed into lacunae of preexisting bone. The relationship of bone and tumor cells is such as to indicate that growth of both occurs simultaneously in order to produce the hyperostosis.

An understanding of the production of new bone with an abnormal pattern and meningioma cells in the interstices depends on the identification of the histologic sequences leading to this result. The histologic picture in hyperostosis cranii can be harmonized with that in the soft tissue tumor if it is shown that the meningioma on reaching the periosteum stimulates a stroma from that source and that this stroma, derived from tissue normally bone producing, continues to produce bone when incorporated in the tumor. This interpretation, presented and amplified by Phemister²¹ in 1923, has not been given much attention in most of the recent studies on meningiomas. The view has been

19. Cushing, H.: *Arch. Neurol. & Psychiat.* **8**:139, 1922.

20. Penfield, W. G.: *Surg., Gynec. & Obst.* **45**:178, 1927. This paper discusses fully the earlier theories on the nature of hyperostosis cranii.

21. Phemister, D. B.: *Arch. Surg.* **6**:554, 1923.

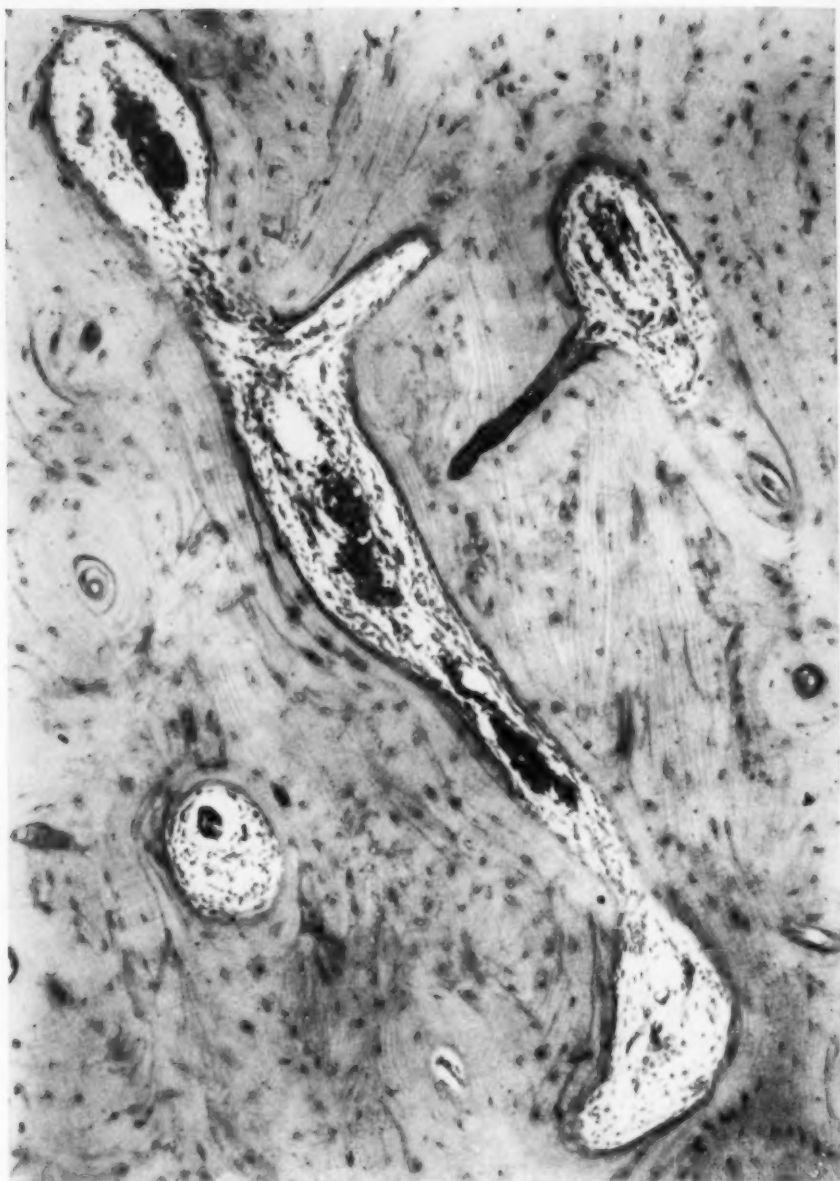


Fig. 6.—A portion of a hyperostosis associated with a meningioma. The mosaic pattern of the bone is wholly irregular. In the lacunae there are deeply stained tumor cells, separated from the bone matrix by a layer of fibrous tissue cells. This alteration of bone mosaic indicates that new bone has been formed in response to the tumor; that the tumor has not been squeezed into preexisting lacunae. The fibrous layer between the tumor cells and the bone matrix is responsible for the formation of bone and not the tumor cells themselves. The overgrowth of bone results from the presence in the meningioma of an ossifying stroma derived from the periosteum. Hematoxylin and eosin; magnification, 95 diameters.

largely overshadowed by the great influence of Mallory's and Penfield's conclusions that the meningioma is a tumor of fibroblastic origin.^{1a,c} It is difficult to see how a fibroblastoma could stimulate an ossifying stroma. Other objections to Phemister's interpretation were summarized by Kolodny.²² He stated: "If the new bone grows out of the old bone, it can not represent 'ossified stroma of the invading endothelioma.' Then again, if one admits that the stroma of the cranial portion of the tumor does ossify, how is it that the intracranial portion, and for that matter also the extracranial portion of the tumor, after the latter perforated the skull, does not ossify to any appreciable extent?" These objections can be answered by a study of this series of cases and of observations in the literature.

The intracranial portion of the meningioma has been described as a tumor of differentiated cells with a stroma of collagenous tissue, blood vessels and, at times, elastic tissue. As the meningioma expands, it continues to derive its stroma from such sources as it encounters, instead of carrying the supporting tissue along from the site of origin of the tumor. The sequences which occur when the periosteum, normally a bone-forming tissue, is reached seem best explained by regarding this layer as being stimulated to growth so as to form an integral part of the stroma of the developing meningioma.

This problem may be approached by comparing the hyperostoses of meningiomas with the osteoplastic metastases of certain carcinomas of the prostate, thyroid and other organs. The epithelial cells from which these carcinomas arise are dependent on a stroma for nutrition and support; they stimulate its production at the primary site of the tumor and in the metastases. Now in the metastases of such carcinomas to bone there are two well recognized types of reaction on the part of the osseous tissue: either the bone is destroyed by the tumor or it is stimulated to produce new bone. The tumors resulting in destruction of bone without formation of new bone are rapidly growing neoplasms which may have stimulated part of their stroma from the periosteum but which have grown so rapidly that there has been little if any bone production. On the other hand, more slowly growing carcinomas deriving their stroma from the periosteum grow sufficiently slowly so that the advancing tissue from the periosteum forms bone, just as it does in its normal position. The mosaic is altered, however, because the lines of growth follow the irregular pattern of the tumor. The result is a bone-productive lesion, a "hyperostosis," which consists of bone of bizarre pattern with carcinoma cells in its interstices.

This situation, in which the growth of bone is clearly due to the participation of the periosteum in the formation of the tumor stroma,

22. Kolodny, A.: Surg., Gynec. & Obst. **48**:231, 1929.

may be directly compared with that of the hyperostosis of the meningioma. Figure 7 shows an area in a region of hyperostosis. In the center of the figure there is a cluster of meningioma cells, the cells being closely applied to one another so as to form a compact, if somewhat asymmetric, whorl. At the periphery of the drawing is an area of bone.

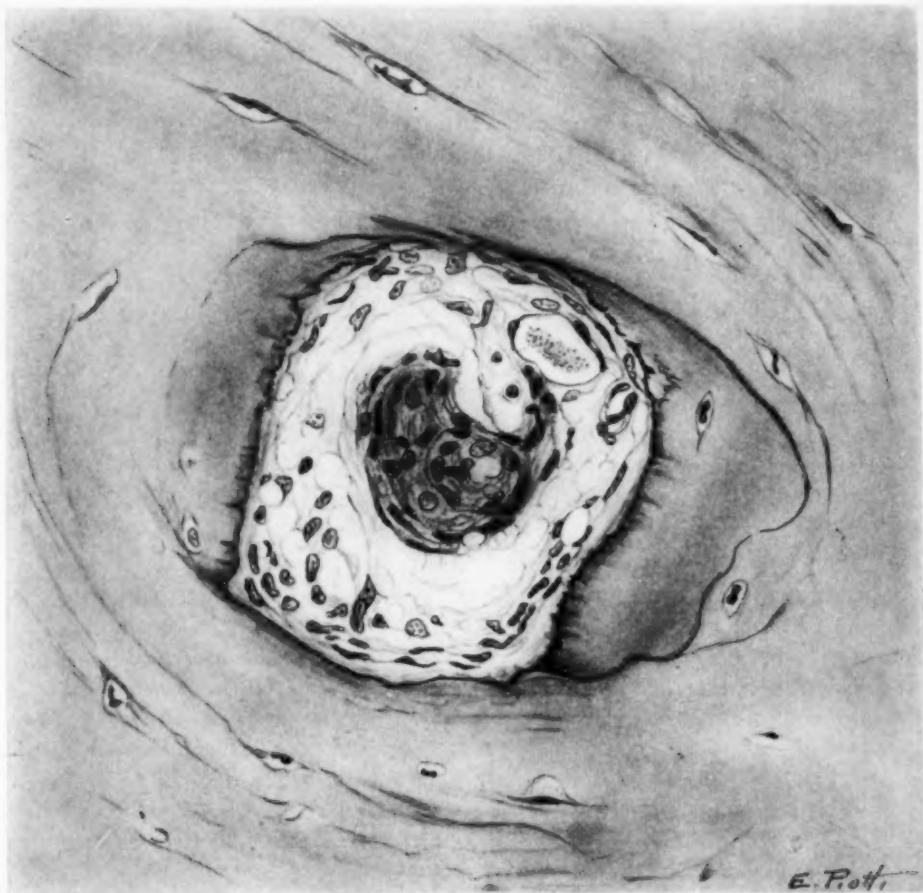


Fig. 7.—An area of a hyperostosis associated with a meningioma. At the center is a cluster of tumor cells of arachnoid cell type. These are separated from the bone matrix by a layer of fibrous tissue cells. Bone formation is due to the fibrous tissue cells and not to the tumor cells. This is a camera lucida drawing of a section stained with hematoxylin and eosin, at a magnification of 760 diameters.

A layer of cells resembling those of the periosteum in all respects except position lies between the bone matrix and the whorl of the meningioma cells, which do not come into contact with the bone matrix at any point.

Penfield²⁰ called attention to such a layer and illustrated it. He commented on the histologic similarity of the cells of this layer and those concerned with "ordinary bone regeneration." He suggested that the layer might have grown in with the tumor to assume osteogenesis or that the meningioma cells themselves might have undergone some transformation and become bone-forming cells. In developing his view of the meningioma as a fibroblastic tumor he did not regard this layer as stroma but stated clearly that the cells of this layer and not those at the center were the cells responsible for the formation of the bone matrix. There were no transitional forms between the meningioma cells and the cells next to the bone matrix, as might have been expected if the tumor cells had undergone a transformation to fit them for bone production. It seems, however, that the relationship of cells and bone matrix in figure 7 is exactly that of a tumor with an ossifying stroma. It is to be emphasized that such typical areas occur only from place to place in a hyperostosis; these areas can be found in all cases after some search. There are many lacunae filled only with fibrous tissue. The amount of osseous stroma stimulated by a small number of meningioma cells may be very great.

When areas from the hyperostoses of meningiomas, such as that shown in figure 7, are compared with those of osteoplastic metastases of carcinomas, the relations are found to be identical—an area of neoplastic cells surrounded by bone matrix with a layer of bone-forming cells between the two. The similarity of the two forms of bone production associated with tumor growth is striking. The differences which may be found lie in the fact that the meningioma is a more slowly growing tumor than most carcinomas and has little tendency to destroy its own stroma as it progresses. The slow growth of the meningeal tumor allows for the formation of the very large hyperostosis which is associated with the long-standing meningioma and which may attain a considerably greater size than do the osteoplastic metastases of carcinomas. Phemister²¹ compared the general pattern of bone growth in hyperostoses and in osteoplastic metastases of carcinomas. He illustrated spiculation in a metastasis from a carcinoma of the prostate. Such spiculation indicates extension of the bone-forming stroma through the tumor and is strikingly similar to the so-called "sunburst" appearance of certain meningiomas in the roentgenogram.

That the similarity between the hyperostosis due to meningioma and that due to metastatic carcinoma may extend even to a clinical resemblance was emphasized in a case discussed by Cushing and Eisenhardt.²³ A hyperostosis of the convexity of the skull was regarded as meningiomatous until supravital examination of excised tissue showed it to be an osteoplastic metastasis of a carcinoma.

23. Cushing and Eisenhardt,^{2c} p. 492.

It has been pointed out by many writers on meningiomas²⁴ and mentioned in a foregoing paragraph that bone extension in the hyperostosis may be outward from the outer table of the skull or inward or, for a short distance, laterally. When the stroma grows outward with the tumor from the outer layer of the periosteum, the bone produced constitutes an external protuberance. Since the direction of tumor growth is chiefly outward, the maximum mass of hyperostosis is often external to the skull. In other cases the hyperostosis extends downward from the skull into the soft tissue part of the tumor. In these cases the tumor reaches the inner layer of periosteum and carries it laterally and downward so as to form a mass of bony tissue and tumor projecting downward from the adjacent uninvolved skull. Combinations of these developments are the rule rather than the exception.

A very few cases have been noted in which the involvement of the adjacent osseous tissue by a meningioma does not result in laying down of bone, the tumor extending directly through the skull and into the overlying muscle. There are at least two possible explanations. It is possible that the tumor is growing too rapidly for the tumor stroma to carry out the function which is the normal property of its constituent cells. It is also possible that the entire stroma of the tumor is derived from sources other than the periosteum as the result of some unusual relationship between the expanding tumor and the overlying bone. From the cases reported in the literature a decision between these possibilities or some further mechanism cannot be made. There were no such tumors in the group on which this study is based.

Bone formation regularly ceases when the tumor is allowed to extend into the tissues of the scalp for such a distance that the bone-forming tissues are left far behind. This offers further confirmation of the theory that the meningioma derives its stroma from the tissues it invades and that these tissues when acting as stroma continue to produce the same types of intercellular material as they do under normal conditions.

Kolodny²² suggested that the proliferation of bone is preceded by local dilatation of the vascular channels in the skull and that the production of new bone associated with infiltration of the skull by tumor cells is a defensive reaction of the skull to the slowly progressive dilatation of the blood vessels. Tumors stimulate the growth of new blood vessels, as they must if their nutritional demands are to be met. Dilatation of blood vessels is found in the regions surrounding a large number of malignant tumors and certain benign tumors. It may be due to pressure on veins with consequent stasis or to increase in the capillary bed supplied by an artery entering the region. These changes are all

24. Cushing and Eisenhardt,²⁰ Penfield²⁰ and others.

of secondary character, and several of them are probably operative in bringing about the initial vascular dilatation in the bone about a meningioma. They do not account for the relationship of tumor cells, connective tissue cells and bone matrix which characterizes hyperostosis cranii.

For the greater incidence of hyperostosis in meningiomas of the parasagittal and pterional regions no entirely satisfactory explanation is available at the present time. Cushing and Eisenhardt^{2c} pointed out that the bregma, pterion and lambda were once fontanelles and that four suture lines meet at the bregma and pterion and three at the lambda. They feel that diastasis of sutures may follow slight blows on the head with resultant contusion of the dura and perhaps stimulation of the arachnoid villi. The high incidence of trauma to the head in patients with meningioma is well known.²⁰ The spinal meningioma apparently does not produce a hyperostosis. Cushing and Eisenhardt^{2c} had no instance in their experience and knew of no description of such a process. Sosman²⁵ had not seen an example. Changes in the vertebrae, such as localized areas of atrophy, are frequently seen; there is no bone production, however. Ariëns Kappers²⁶ pointed out, from a study of the comparative anatomy of the meninges, that in the adult the periosteal membrane of the dura is fused with the internal layer in the cranial cavity but that it is better not to consider the periosteal membrane of the spinal dura a part of the dura proper. This difference in anatomic relation suggests that the spinal meningioma derives all its stroma from the inner layer of the dura, which is not bone forming, and that the fusion of the two parts of the dura in the cranial cavity may have something to do with the accessibility of the bone-forming layer for the stroma of the intracranial meningioma at any of the favored sites of origin of those with hyperostoses.

ISOLATED BONE PLAQUES IN THE MENINGIOMA

In certain meningiomas there are areas of bone which are not in direct continuity with the skull or any other source of tissue normally bone producing. When the space separating such an area of bone from a region of hyperostosis is small, it is possible that the continuity of the bony stroma has been interrupted by further growth of the tumor, thus isolating bone-containing tissue. However, in certain meningiomas the isolation is complete. One such tumor occurred in the present series. Are these, then, indications that the cells of the tumor under certain circumstances are capable of forming bone? The evidence seems to indicate that the contrary is the case. In the first place, it should be

25. Sosman, M. C.: Personal communication to the author.

26. Ariëns Kappers, C. U.: *Arch. Neurol. & Psychiat.* **15**:281, 1926.

pointed out that plaques far removed from the periosteum present an important difference from the bone formed in the hyperostosis. The interstices of the bone are filled only with fibrous tissue; there are no meningioma cells. Thus the characteristic arrangement of cells and bone seen in the hyperostosis is absent. Whether this is invariably the case cannot be determined from the material on this point now available. It is apparent that bits of tumor may appear included within a bit of metaplastic bone, but the relationships emphasized in hyperostoses are absent. The lack of tumor cells within the plaque suggests that the latter may result from ossification of a previous calcareous deposit rather than from action of tumor cells. Calcification is frequent in meningiomas and also in the arachnoid membrane itself.²⁷ Cushing and Weed²⁷ pointed out that calcification in arachnoid cells is preceded by proliferation of these cells. The evidence tends to show that the lime salts are laid down within the cell bodies, bodies apparently partially degenerated. Wells²⁸ emphasized that the degree of vascularity is of importance in the formation of calcareous and osseous deposits. Calcium cannot enter the area if there is no vascular supply, for it has no means of entrance. If the vascularity is too great, the necrotic materials are carried away before calcification occurs. Weed⁵ pointed out that the conditions required for calcification to take place are met in the meningeal clusters. Much the same conditions obtain in many meningiomas.

It is a general histologic sequence that fibroblasts in contact with calcareous material can produce ossification. This is a familiar phenomenon in arteriosclerotic blood vessels, in chronically inflamed tonsils and in many other situations. While Wells²⁸ was unable to state the nature of the immediate stimulus, he expressed the belief that there is no essential difference between the process of normal ossification and that of the pathologic deposits. He stated that the conditions necessary to the formation of a pathologic bone deposit are the presence of calcium and the presence of fibroblasts. The sequence leads from necrosis of tumor cells to deposition of calcium and finally to ossification by fibroblasts. Since fibroblasts are necessary for ossification, it might be suggested that the ossification argues for the fibroblastic origin of meningiomas. Yet fibroblasts are present in the scheme here suggested even though their role is a secondary one. It has been indicated earlier in this paper that in the psammoma bodies the stromal cells tend to persist after necrosis of the cells of arachnoid cell type. This is by no means uncommon in tumors with stroma arising in other organs.

27. Cushing, H., and Weed, L. H.: *Bull. Johns Hopkins Hosp.* **26**:367, 1915.

28. Wells, H. G.: *Arch. Int. Med.* **7**:721, 1911.

That such isolated bone plaques represent ossification of areas of necrosis receives confirmation from a report by Weiser.²⁹ The first case presented in his paper is one in which a meningioma was removed at operation. Thirteen years later there was a recurrence. When this tumor was removed, there was a centrally placed plate of bone of large size at its center. This was not attached to the skull. Weiser interpreted this as indicating that a portion of the primary tumor was left extradurally at the first operation. This tumor fragment, impaired in vitality and nutrition, provided a hyaline matrix for calcification and secondary ossification.

MENINGIOMAS PASSING THE PIAL BARRIER

Evidence has been presented that collagenous tissue, elastic tissue and osseous tissue may participate in the stroma of meningiomas. Can supporting cells of the central nervous system also be incorporated in the stroma? It is only in rare instances that a meningioma breaks through the pial barrier and invades the substance of the underlying brain.^{2b, c} There was such an instance in the present series: a meningioma of the upper cervical portion of the spinal cord invaded the underlying spinal cord tissue. There were whorls of cells of arachnoid cell type among the fiber tracts for a short distance from the pial border. No stimulation of astrocytes as supporting tissue could be found. The meningioma in this respect resembled metastatic carcinoma of the central nervous system, which does not stimulate ectodermal cells to participate in its stroma. Astrocytes are, of course, increased in the region outside a meningioma where the tumor affects the brain or spinal cord by pressure. Such an increase is the usual reaction of these tissues to pressure and can be brought about as easily by compression due to the presence of inert foreign material as by a meningioma.

THE MECHANISM OF DURAL ATTACHMENT

It has already been pointed out that the meningioma in most instances arises from the pacchionian granulations which are within the dura; hence a dural attachment is present from the beginning. It is not necessary to suppose that this is always the case. There is a mechanism for participation of the dura in very small lesions of the arachnoid. Lear and Harvey,¹⁸ in experiments previously discussed, showed that healing of interruptions in the arachnoid surface without injury to the dura is accompanied by participation of connective tissue and blood vessels of the undersurface of the dura. Arachnoid cells tend to form groups and clusters;⁸ these develop in the course of time into small elevations attached to the arachnoid. It is probable that when the cell clusters

29. Weiser, A.: *Deutsche Ztschr. f. Chir.* **192**:405, 1925.

become somewhat larger they may produce sufficient alteration of the arachnoid for the dura to participate in the nodule. It has been shown that even the amount of irritation caused by a silk suture rubbing against otherwise normal arachnoid results in sufficient change in the meninges to cause connective tissue and blood vessels to grow out from the dura.¹⁵ In the case of the arachnoid cell cluster the tissues from the dura form a large part of the supporting and nutritive stroma of the nodule. The new blood vessels from this source would be especially important because of the paucity of the blood supply of the arachnoid. In this way one may picture the initial steps of the formation of the meningioma unassociated with pacchionian granulations. Where is the dividing line between the arachnoid cell cluster and the very small meningioma seen incidentally at necropsy? The difference seems to depend on the participation of tissue from the dura and the histologic sequences which follow.

This mechanism for dural attachment is by no means confined to the meningioma. It has been discussed as the means by which there comes to be a dural attachment of the glioma which arises from a neuroglial nest in the leptomeninges,³⁰ first described by Wolbach.³¹ In this tumor and in the glioma arising in the substance of the brain which reaches the surface (notably glioblastoma multiforme) there is an attachment to the dura. Collagen and blood vessels grow into these tumors from the pachymeninx; they are especially easy to follow in those tumors because the other tissues are of glial origin except for a capillary network and its few accompanying collagen fibers.³² Kolodny²² stated that the essential difference between meningiomas (which produce hyperostoses) and tumors which do not is in the blood supply, that of gliomas being cerebral and that of meningiomas, meningeal. This difference results from the anatomic situation of the tumor in its early stages and is not related to hyperostosis. It has also been shown that blood vessels from the dura can enter the glioma, at least to a limited extent, without any changes in the overlying bone.

RELATION OF THE MENINGIOMA TO THE NEUROFIBROMA

It is interesting to arrange a series of meningiomas so that they progress from the most calcified and fibrous at one end to the most cellular at the other. The meningiomas most often illustrated in textbooks are those at the former end of the series. As the more cellular types are approached, there is a certain resemblance to those neurofibromas in which palisading is least marked. That this resemblance is

30. Bailey, O. T.: *Arch. Path.* **21**:584, 1936.

31. Wolbach, S. B.: *J. M. Research* **16**:495, 1907.

32. Bailey,³⁰ fig. 6.

not entirely superficial is suggested by the cases in which multiple neurofibroma and multiple meningioma coexist.^{2c} It is beyond the province of this paper to discuss the nature of the neurofibroma other than to point out that Penfield's findings^{1c} in regard to the distribution of collagen as the determining factor in the formation of the palisades may be of use in the explanation of the similarity. Embryologic homologies of arachnoid cells and sheath cells may also be involved. This matter is one for further research and involves a clarification of embryologic facts now obscure.

COMMENT

In this paper the meningioma has been considered as a specific tumor arising from differentiated arachnoid cells and possessing a complicated stroma. The growth sequences described indicate the manner in which histologically diverse pictures are built up from the same materials. These differences in histologic appearance, then, are superficial and become resolved by further study. The term "meningioma" does not adequately describe this tumor but has the advantage of being widely used. On a strictly logical basis, "arachnobloma" would be a proper name for the tumor, but it seems inadvisable to replace a convenient familiar term with a more cumbersome one in naming this relatively common lesion. Of the other names applied to this tumor, none is less appropriate than "dural endothelioma." "Dural endothelioma" indicates an origin from a cell which does not exist in a membrane associated with the tumor only secondarily and sometimes not at all.

The consideration of the meningioma as a specific tumor is by no means new. In view of this fact it is unfortunate that numerous recent publications dealing with meningiomas have proposed dividing them into a great number of special classes and have included in the classification meningeal tumors of most diverse natures. It would seem to serve no useful purpose to classify such lesions into different divisions based only on kind or extent of degeneration or on differences arising from variations in relations during growth. One of the tumors sometimes included in these elaborate classifications of meningiomas is the chondroma. This certainly has no relation to the meningioma discussed in this paper other than that of location. The chondroma arises not from arachnoid cells but from an embryonic rest, one source of which may be the neural crest, since cartilage is among its derivatives.³³ Certainly the lipomas of the meninges should not be included among the meningiomas. Whatever the source of the fat in these tumors, it is certain that they are not related to the tumors of arachnoid cell origin. It is also unfortunate that the term "angioblastic meningioma" has

33. Landacre, F. L.: *J. Comp. Neurol.* **33**:1, 1921.

been applied to certain blood vessel tumors which originate in meningeal regions. These tumors resemble hemangiomas with secondary changes well known in hemangiomas of the skin and certain viscera. They are not tumors of arachnoid cells.

This insistence on the specific nature of the meningioma has implicit in it the feeling that the arachnoid cell is also specific and cannot change indifferently into an angioblast, a fibroblast or another cell as some writers have suggested. While it is clearly recognized that certain variations of form, of speed of growth and of arrangement can and do occur, there are limits beyond which these changes do not go. In tumors, especially if a series can be studied, transition stages should be found between various histologic appearances of the type cell. For such extensive alterations as those suggested by other writers, one would have to postulate an origin of the meningioma in a multipotential cell. From the evidence at hand, however, the meningioma seems best interpreted as arising from a differentiated cell which maintains this differentiation in the tumor to which it gives rise. A recognition of the histologic sequences is essential in studying the types of tissue participating in a tumor and the mechanism by which its variants are created.

SUMMARY

The meningioma is a tumor arising from the arachnoid cell, either in the arachnoid villi or on the surface of the arachnoid. Neither the ectodermal theory of the French neuropathologists nor the fibroblastic theory of Mallory in regard to the origin of this tumor is sufficient to explain completely the variations in histologic structure encountered in a series of these neoplasms.

The arachnoid cell is differentiated and is specialized as a covering cell. The differentiation persists in meningiomas with the result that the neoplastic cells require a supporting and nutritive stroma. Consideration of the sequences of growth shows that the arachnoid cells retain their covering property in the tumors; they cover intercellular fibers, blood vessels or themselves, with the result that whorls are built up.

The stroma of meningiomas consists of several elements, the most constant of which are collagenous connective tissue and blood vessels. The fibroglia fibrils seen in meningiomas belong to the fibroblasts of the stroma and not to the neoplastic cells. Elastic tissue also forms part of the stroma of some meningiomas.

Hyperostosis cranii is produced by involvement of the skull by the meningioma. It develops when the stroma of the meningioma is derived from the periosteum. An ossifying stroma is then produced which gives rise to a mass of bony tissue with tumor cells in the interstices.

Bone plaques not connected with the periosteum occur occasionally in meningiomas. These plaques result from ossification of areas of degeneration in the tumors.

A mechanism has been described by which leptomeningeal tumors secure a dural attachment.

The great number of histologic variants of the meningioma depends on relationships of the neoplastic cells, with their tendency to cover whatever they touch, to a complex stroma, which continues to form intercellular materials similar to those which the component parts form under normal circumstances. The term "arachnoid blastoma" describes these tumors more accurately than the term "meningioma," but the latter is convenient and familiar. The term, however, should be restricted to tumors arising from arachnoid cells and not extended to include neoplasms which have little in common with them except location.

SUBCUTANEOUS NODULES OF RHEUMATOID ARTHRITIS AND RHEUMATIC FEVER

A PATHOLOGIC STUDY

GRANVILLE A. BENNETT, M.D.

J. WALLACE ZELLER, M.D.*

AND

WALTER BAUER, M.D.

BOSTON

The subcutaneous nodule of rheumatoid arthritis has been the subject of numerous investigations,¹ which have made known many of its clinical and pathologic characteristics. Its exact significance and genesis remain uncertain. This is also true of the previously described² and more thoroughly studied³ subcutaneous nodule of rheumatic fever.

Comparative studies⁴ have shown that these nodules are very similar if not identical in many of their clinical and pathologic features. It has been maintained^{1*} that the observed similarities represent different

* Nemours Foundation Fellow, 1938, 1939.

This is publication 46 of the Robert W. Lovett Memorial for the study of crippling disease, Harvard Medical School.

The expenses incurred in this study have been defrayed in large part by grants from the Commonwealth Fund to the Robert W. Lovett Memorial and the House of the Good Samaritan.

From the departments of pathology and medicine of the Harvard Medical School, the medical clinic of the Massachusetts General Hospital and the Massachusetts Department of Public Health.

1. (a) Pitt, G. N.: *Brit. M. J.* **2**:1324, 1893; (b) *Tr. Clin. Soc. London* **27**: 54, 1893-1894. (c) Hawthorne, C. O.: *Studies in Clinical Medicine*, London, John Bale, 1912. (d) Freund, E.: *Wien. Arch. f. inn. Med.* **16**:73, 1928. (e) Dawson, M. H., and Boots, R. H.: *J. A. M. A.* **95**:1894, 1930. (f) Clawson, B. J., and Wetherby, M.: *Am. J. Path.* **8**:283, 1932. (g) Dawson, M. H.: *J. Exper. Med.* **57**:845, 1933. (h) Collins, D. H.: *J. Path. & Bact.* **45**:97, 1937.

2. Wells, W. C.: *Tr. Soc. Improv. M. & Chir. Knowl.* **3**:373, 1812. Meynet, P.: *Lyon méd.* **19**:495, 1875. Barlow, T., and Warner, F.: *Tr. Internat. M. Cong.*, London **4**:116, 1881.

3. (a) Hirschsprung, H.: *Jahrb. f. Kinderh.* **16**:324, 1881. (b) Cavafy: *Brit. M. J.* **1**:622, 1883. (c) Swift, H. F.: *J. Exper. Med.* **39**:497, 1924. (d) MacCallum, W. G.: *J. A. M. A.* **84**:1545, 1925. (e) Mote, J. R.; Massell, B. F., and Jones, T. D.: *J. Clin. Investigation* **16**:129, 1937. (f) Keil, H.: *Medicine* **17**:261, 1938.

4. (a) McEwen, C.: *Arch. Path.* **25**:303, 1938. (b) Hawthorne.^{1c} (c) Dawson.^{1*}

phases of the same fundamental process, lending support to the theory that rheumatoid arthritis and rheumatic fever are different responses to the same etiologic agent. Klinge and Grzimek⁵ came to the same conclusion after an extensive study of the pathologic changes of the two diseases. More recently Collins^{1b} described distinguishing features of these subcutaneous lesions and concluded that there was insufficient evidence to justify postulating either a close pathologic relationship or a common etiologic agent.

During the past ten years a large number of subcutaneous nodules have been obtained from patients with rheumatoid arthritis and from subjects with rheumatic fever. A detailed comparative study of these lesions was undertaken in order to describe their characteristic features and determine, if possible, whether they are disease specific. It was further hoped that such an investigation might throw some light on their genesis and thereby aid in the search for the causative agent or agents.

MATERIALS AND METHODS

Sixty-seven subcutaneous nodules were removed from 44 patients with rheumatoid arthritis. Dr. Tracy B. Mallory, of the Massachusetts General Hospital, allowed this material to be placed at our disposal. Additional nodules (790) were obtained post mortem from the sclera, pericardium, pleura and synovial tissue of the patient in this group from whom specimen 664 (table 1) was obtained during life. Similar lesions were found in the sclera (specimen 476) and synovial tissue (specimen 730) of 2 other patients. An additional subcutaneous nodule (880) was obtained from a child whose disease was more like rheumatoid arthritis than rheumatic fever. Nineteen subcutaneous nodules were obtained from 15 subjects of rheumatic fever who had rheumatic heart disease. Three of the patients with rheumatoid arthritis had heart disease that was probably of rheumatic origin. The material from the subjects with rheumatic fever consisted in part of prepared microscopic sections that have been described previously.^{3e} Dr. T. Duckett Jones, of the House of the Good Samaritan, supplied this material and cooperated with us in securing additional specimens.

A few selected specimens were examined bacteriologically by various cultural methods, and portions of others were used for animal inoculation. The majority of the specimens were divided into portions suitable for fixation in 10 per cent neutral formaldehyde solution and Zenker's fluid. Regaud's solution and a solution of formaldehyde U. S. P. plus alcohol were sometimes used as fixatives. The blocks of tissue were embedded in paraffin and sectioned at 6 to 8 microns. Sections from each specimen were stained in most instances by the following technics: Giemsa, eosin-methylene blue, hematoxylin-eosin, phosphotungstic acid-hematoxylin (Mallory) and aniline blue (Mallory). Occasionally preparations were stained for the demonstration of reticulum or by the Levaditi and the Gram method.

The sections from each specimen were examined microscopically, described in detail and the condition diagnosed without knowledge of the clinical studies. The sections were then restudied without regard to the type of disease or the duration of the lesion. The structural and cytologic alterations noted were graded as to

5. Klinge, F., and Grzimek, N.: *Virchows Arch. f. path. Anat.* **284**:646, 1932.

TABLE 1.—Nodules from Patients with Rheumatoid Arthritis and Rheumatic Fever

Specimen* No.	Age of Pa- tient Yr.	Sex	Clinical Diagnosis	Duration of Disease Yr. Mo.	Nodule Examined			Central Zone (Zone of Degeneration)					Peripheral Zones					Diagnosis Based on Examination of Nodules		
					Loca- tion	Size, Mm.	Approx- imate Dura- tion, Days	"Fibrinoid" Change		Complete Necrosis and Degeneration		Intermediate Zone (Cellular)			Diffuse Cellular Infl.	Focal and Prolif- erative Changes in Blood Vessels	R.F. R.A.			
								Swelling	Lattices	Small Focal Areas	Large Con- glomer- ate Areas	Cavity Forma- tion	Pallid- ing of Cells	Cellu- larity					Multinu- cleated Cells	Mitotic Figures
Nodules under 1 month in age	24	9	M	R.F.-R.H.D.	6	Elbow	7 x 6	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	70	10	M	R.F.-R.H.D.	6	Knee	5 x 3	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	7	16	F	R.F.-R.H.D.	2	Wrist	6 x 3	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	57	9	M	R.F.-R.H.D.	2	Elbow	5 x 4	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	833a	32	M	R.F.-R.H.D.	10	Scapula	30 x 20	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	75	10	M	R.F.-R.H.D.	6	7 x 7	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	157	6	M	R.F.-R.H.D.	5	Elbow	11 x 5	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	833b	32	M	R.F.-R.H.D.	10	Scalp	10 x 10	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	83	12	F	R.F.-R.H.D.	5	4 x 3	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	71	10	M	R.F.-R.H.D.	5	Elbow	7 x 6	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	73	12	F	R.F.-R.H.D.	10	8 x 8	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	659	21	F	R.F.-R.H.D.	1	Elbow	9 x 7	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	682	43	M	R.A.	6	20 x 10	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	680	43	F	R.A.	11	Elbow	23 x 20	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	615	11	F	R.A.	2	Elbow	10 x 8	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
595	31	M	R.A.-R.H.D.	8	Elbow	10 x 5	+++	+++	+	+	0	0	+++	+	+	+	+	+	+	+
	629	43	M	R.A.	7	Toe	17 x 6	+++	+++	+	+	0	0	+++	+	+	+	+	+	+
	623	55	M	R.A.	6	Elbow	10 x 5	+++	+++	+	+	0	0	+++	+	+	+	+	+	+
Nodules 1 month to 6 months in age	82	6	M	R.F.-R.H.D.	4	Elbow	6 x 5	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	97	10	F	R.F.-R.H.D.	4	Elbow	9 x 7	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	3	12	F	R.F.-R.H.D.	5	3 x 3	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	52	14	F	R.F.-R.H.D.	4	Scalp	5 x 3	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	579	32	F	R.F.-R.H.D.	11	Elbow	6 x 4	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	79	23	F	R.F.-R.H.D.	14	Elbow	9 x 7	++++	++++	+	0	0	+	+++	+	+	+	+	+	+
	2	10	F	R.F.-R.H.D.	2	Elbow	6 x 3	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	777	56	M	R.A.	1	Elbow	10 x 9	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	801	53	F	R.A.	1	Ankle	6 x 5	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	664	55	M	R.A.	2	Knee	8 x 4	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	512	33	M	R.A.	2	Elbow	20 x 20	++++	++++	+	+	0	+	+++	+	+	+	+	+	+
	52	28	F	R.A.	6	Elbow	5 x 3	++++	++++	+	+	0	+	+++	+	+	+	+	+	+

magnitude and recorded on a prepared table listing the pathologic changes observed previously. A second diagnosis was made on the basis of these observations. After the results of these two microscopic studies had been compared, a final detailed table was made in which the lesions were arranged according to age. The clinical diagnoses were then recorded for comparison with the pathologic interpretations (see table 1).

Approximately the same number of nodules was removed from each sex. The patients differed markedly in age. The youngest patient with rheumatoid arthritis was 11 years of age; the oldest, 80; the average age for the group was 48 years. The average age for the 15 subjects with rheumatic fever was 14 years; the youngest was 6; the oldest, 32 years. The duration of disease varied greatly, from three months to fourteen years in the subjects with rheumatic fever and from four months to twenty-six years in the patients with rheumatoid arthritis. The nodules of known duration had been present from five days to five months in the rheumatic fever group and eleven days to six years in the group with rheumatoid arthritis.

The nodules varied markedly in size, as is shown in table 2.

The subcutaneous nodules of these two diseases are usually multiple. Thirty-six of the patients with rheumatoid arthritis and 13 of the 15 patients with rheumatic fever had more than one nodule. The frequency with which different areas of the body were affected in each disease is given in table 3.

PATHOLOGIC OBSERVATIONS

The subcutaneous nodules of rheumatoid arthritis and rheumatic fever on microscopic examination are found to consist of single or multiple lesions of varying size. They are alike in that each lesion has three reasonably well defined merging zonal areas (fig. 1). These areas have been designated by others as: (a) the *central zone*, or zone of necrosis; (b) the *intermediate zone*, comprised of proliferating cellular tissues, the cells of which may or may not be regularly oriented (palisading), and (c) the *peripheral zone*, consisting of the inflamed tissues in which the nodule develops.

The more constant pathologic changes observed in each zone have been recorded in table 1 as follows: 0, if absent; +, if present in minimal amounts in any portion of the lesion, and ++ to ++++, according to the extent of the alteration. This method of grading is at best an approximation but does enable one to record the relative degree of any given type of change.

Examination of the central zones of the individual nodules revealed two principal types of pathologic change. These alterations have been designated as (a) "fibrinoid" change and (b) necrosis and degeneration (see table 1). It will be noted that many of the nodules exhibited both. Usually, however, one was decidedly more pronounced than the other, and in only rare instances were the two equally prominent. The "fibrinoid" change was predominant in rheumatic fever; necrosis and degeneration, in rheumatoid arthritis. Widespread separation and swelling of collagen bundles were the chief alterations in the lesions in which

"fibrinoid" changes predominated (figs. 1A and 2). Such collagen bundles stained intensely with the eosin dye. Appropriate staining methods showed that some of the fibrillar components of connective tissue had persisted in many of the swollen, acidophilic, "fibrinoid" strands, indicating that this change was due largely to exudation of plasma and fibrin into and between the collagen fibrils. A characteristic latticed arrangement was observed in lesions exhibiting marked "fibrinoid change" (fig. 2B). The fenestrations in such latticed structures contained fibrin strands, precipitated serum and various types of cells. Among the cells that could be identified, mononuclear leukocytes and lymphocytes predominated, although polymorphonuclear leukocytes, plasma cells and an occasional eosinophil were present. It will be seen from table 1 that these extensive exudative changes as well

TABLE 2.—Size of Nodules

	Rheumatoid Arthritis	Rheumatic Fever
Maximal dimensions.....	40×30 mm.	30×20 mm.
Minimal dimensions.....	5×3 mm.	3×3 mm.
Average dimensions (approximate).....	15×11 mm.	8×6 mm.

TABLE 3.—Physical Areas from Which Nodules Were Obtained

Cases	El-bows	Fingers	Knees	Feet	Spine	Scalp	Wrists	An-kles	Scap-ular Area	Tho-racic Wall
Rheumatoid arthritis...	44	40	9	6	6	3	3	2	2	0
Rheumatic fever.....	15	14	3	5	2	4	2	4	2	0

as the clearly marked "fibrinoid" swelling of collagen were almost always observed in the rheumatic fever nodules. These same specimens frequently contained a few round or oval lesions of the size of miliary tubercles, bearing a distinct resemblance to myocardial Aschoff nodules (fig. 3). One of these smaller lesions may be found in any portion of the nodule. The central areas contain swollen, hyalinized, acidophilic and fragmented collagen. These changes are very similar to those described in great detail by Klinge,⁶ and the term "fibrinoid degeneration" has been generally applied to them. In an occasional nodule sequestration of the altered collagen (fig. 3B) or its disintegration (fig. 3C) was observed.

The nodules of rheumatoid arthritis contained large central areas of well marked necrosis, in which extensive or complete disintegration of all preexisting connective tissue had taken place. Such areas of necrosis,

6. Klinge, F.: Ergebnisse der allgemeinen Pathologie und pathologischen Anatomie des Menschen und der Tiere, Munich, J. F. Bergmann, 1933.

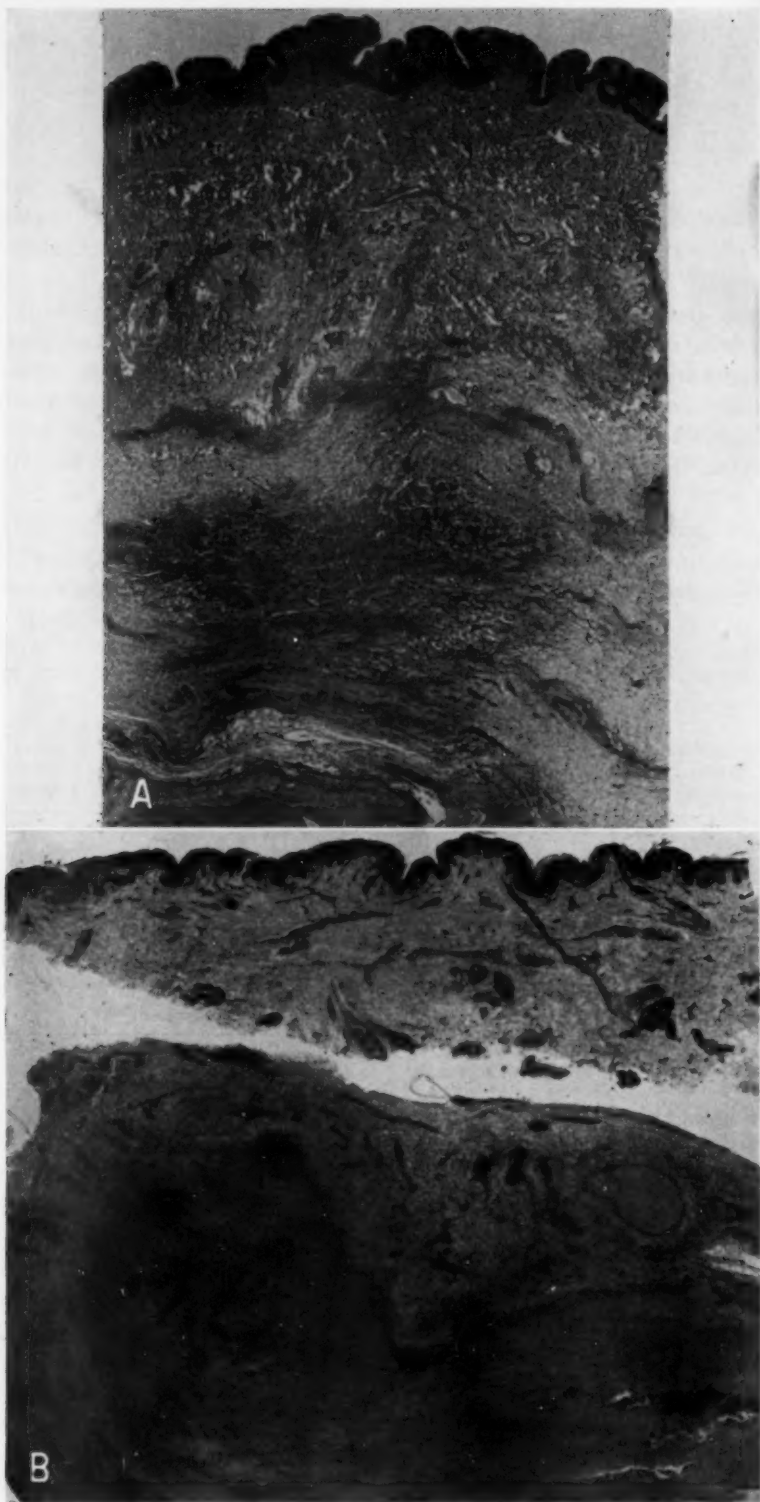


Fig. 1.—*A*, photomicrograph ($\times 8$) showing the greater portion of a ten day old subcutaneous nodule from a patient with rheumatic fever and rheumatic heart disease. Note the diffuse exudative reaction with swelling and separation of the connective tissues. Section stained with hematoxylin and eosin.

B, photomicrograph ($\times 8$) of a subcutaneous nodule of unknown age from a patient with rheumatoid arthritis. The large and small foci of complete necrosis and degeneration of tissue are clearly shown. Note the sharp boundaries between the necrotic zones and the surrounding viable tissues. Section stained with hematoxylin and eosin.

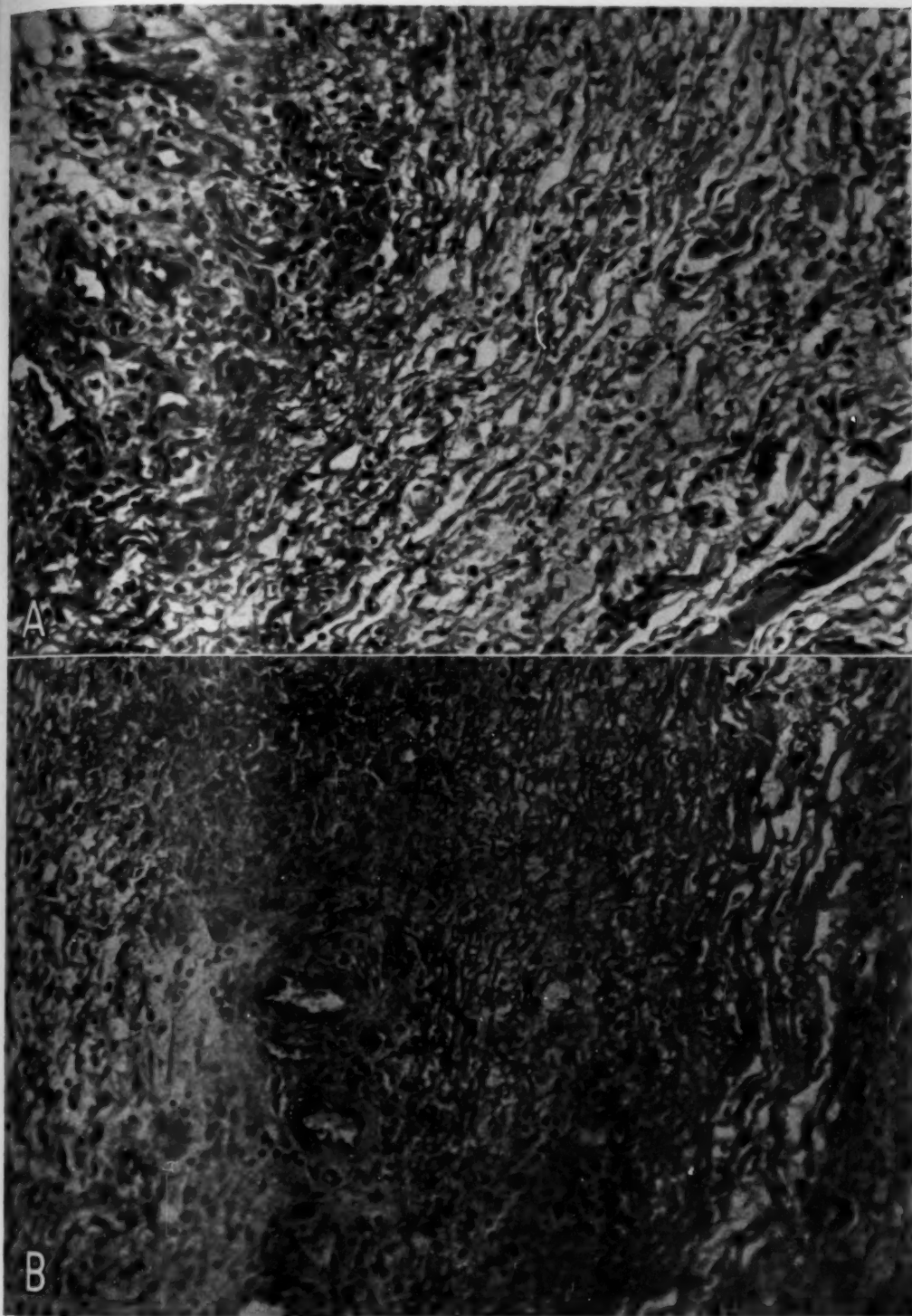


Fig. 2.—*A*, photomicrograph ($\times 198$) showing the interstitial edema and cellular infiltration that prevailed in the rheumatic fever nodule shown in figure 1 *A*. Note the swelling and separation of collagen bundles. Alterations due to inflammation may be seen in the highly vascular tissue to the left.

B, photomicrograph ($\times 198$) of a three month old subcutaneous nodule from a patient with rheumatic fever. A marked exudative reaction with pronounced "fibrinoid" change in collagen is present. There is, however, little evidence of palisading of cells, and the margins of the lesions are not clearly demarcated. Both sections stained with hematoxylin and eosin.

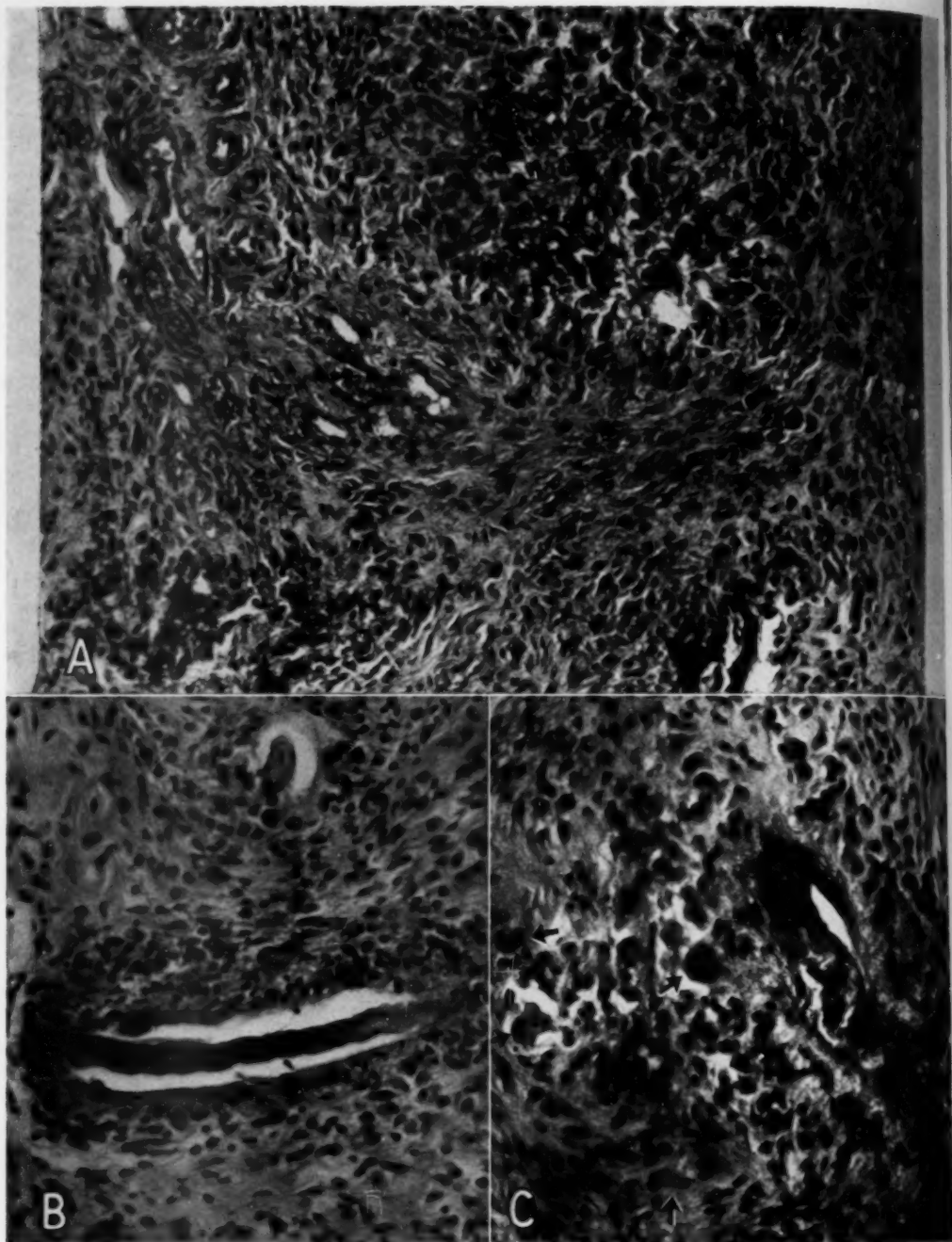


Fig. 3.—*A*, photomicrograph ($\times 190.5$) illustrating the miliary focal lesions that were often observed in the subcutaneous nodules from patients with rheumatic fever. The resemblance of these lesions to myocardial Aschoff nodules is apparent. The nodule from which this photomicrograph was made was obtained from a 16 year old patient and had been present for one week.

B and *C*, changes believed to represent stages in the development of such lesions as are illustrated in *A*. The photomicrograph ($\times 190.5$) on the left shows separation (sequestration) of a bundle of degenerating collagen, accompanied by cellular infiltration and proliferation in the adjacent tissues. The right hand photomicrograph ($\times 212$) shows a further stage in the degeneration of collagen. Note the mitotic figure and the multinucleated cells in the marginal area. The sections reproduced in these photomicrographs were stained by the Giemsa method. They were made from a seven day old rheumatic fever nodule.

while sometimes present as rounded foci of microscopic size, were more frequently large enough to be readily seen macroscopically (fig. 1 *B*). Regardless of size, such foci consisted of masses of granular or structureless debris (figs. 4 and 5 *A*). Remnants of preexisting blood vessels or dense connective tissue structures were often recognized in these necrotic masses (fig. 5 *B*). Such structures were identified readily in preparations stained for the demonstration of collagen. Many of the nodules showed irregularly shaped or slitlike cavities of varying size, whose margins consisted of frayed, necrotic connective tissue. These spaces represented liquefaction, probably resulting from more complete degeneration of the central necrotic zone. The centers of some of the older lesions were calcified, and in 1 instance there was evidence of cholesterol deposition. As previously stated, "fibrinoid" change was rarely observed in such lesions. The exudative reaction, when present, was usually found in the marginal zone of vascular granulation tissue (fig. 6 *B*).

The type of tissue reaction seen in the intermediate or proliferative zone could in most instances be correlated with that of the central area. In the nodules of rheumatic fever it consisted chiefly of loose-textured, edematous, inflamed connective tissue, in which inflammatory cells of the types previously mentioned were evenly distributed (figs. 1 *A* and 2). Numerous large oval or round cells (often multinucleated) containing large vesicular nuclei with prominent nucleoli surrounded by an abundance of nongranular cytoplasm were present. They were most frequently seen in areas showing extensive "fibrinoid degeneration." In such instances they resembled the predominant cells of myocardial Aschoff nodules (fig. 3) and were similar to those which fail to react to neutral red or janus green in supravitaly stained preparations.⁷ Mitotic figures were numerous. They were occasionally seen in fibroblasts and in the endothelial cells of capillaries. Palisading, if present, was minimal.

The intermediate zone of the nodule of rheumatoid arthritis was clearly demarcated. Palisading or radial orientation of cells at the margin of the necrotic zone was a prominent feature of these lesions (figs. 4, 5 and 6). This regular cell alinement appeared as a cellular wall around the centrally placed necrotic area or bursa-like cavity (fig. 6 *A*). The majority of the cells were elongated or oval and showed evidence of active multiplication. The presence of varying amounts of intercellular collagen and reticulum indicated that many of them were fibroblasts. Others resembled the epithelioid cells of a tuberculous lesion. The previously mentioned multinucleated cells were seen frequently. Budding capillaries, most numerous in the outer portion, were observed. The presence of numerous papillary or villi-like processes showing hyaline degeneration indicated that this zone was undergoing periodic or continuous

7. McEwen, C.: *J. Exper. Med.* **55**:745, 1932; footnote 4a.

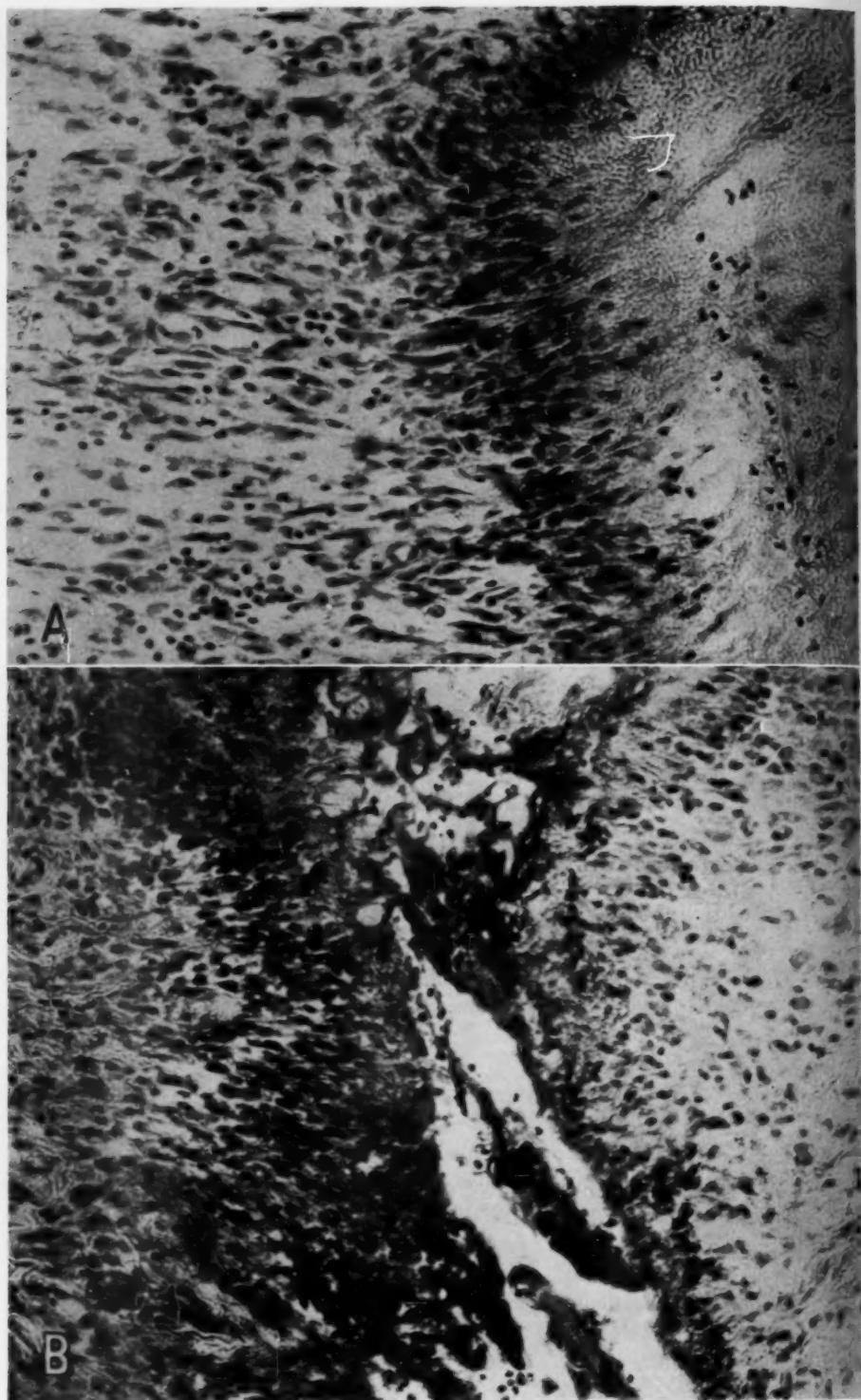


Fig. 4.—*A*, intermediate zone ($\times 230$) of a one month old subcutaneous nodule (specimen 629) from a patient with rheumatoid arthritis. Note the regular orientation of oval and fusiform cells between the connective tissue to the left and the large necrotic mass to the right.

B, a further stage of disintegration of the necrotic tissue in the central zone ($\times 230$) as compared with *A*. The nodule (specimen 615) from which this photomicrograph was taken was obtained from a patient with rheumatoid arthritis and had been present for approximately one month.

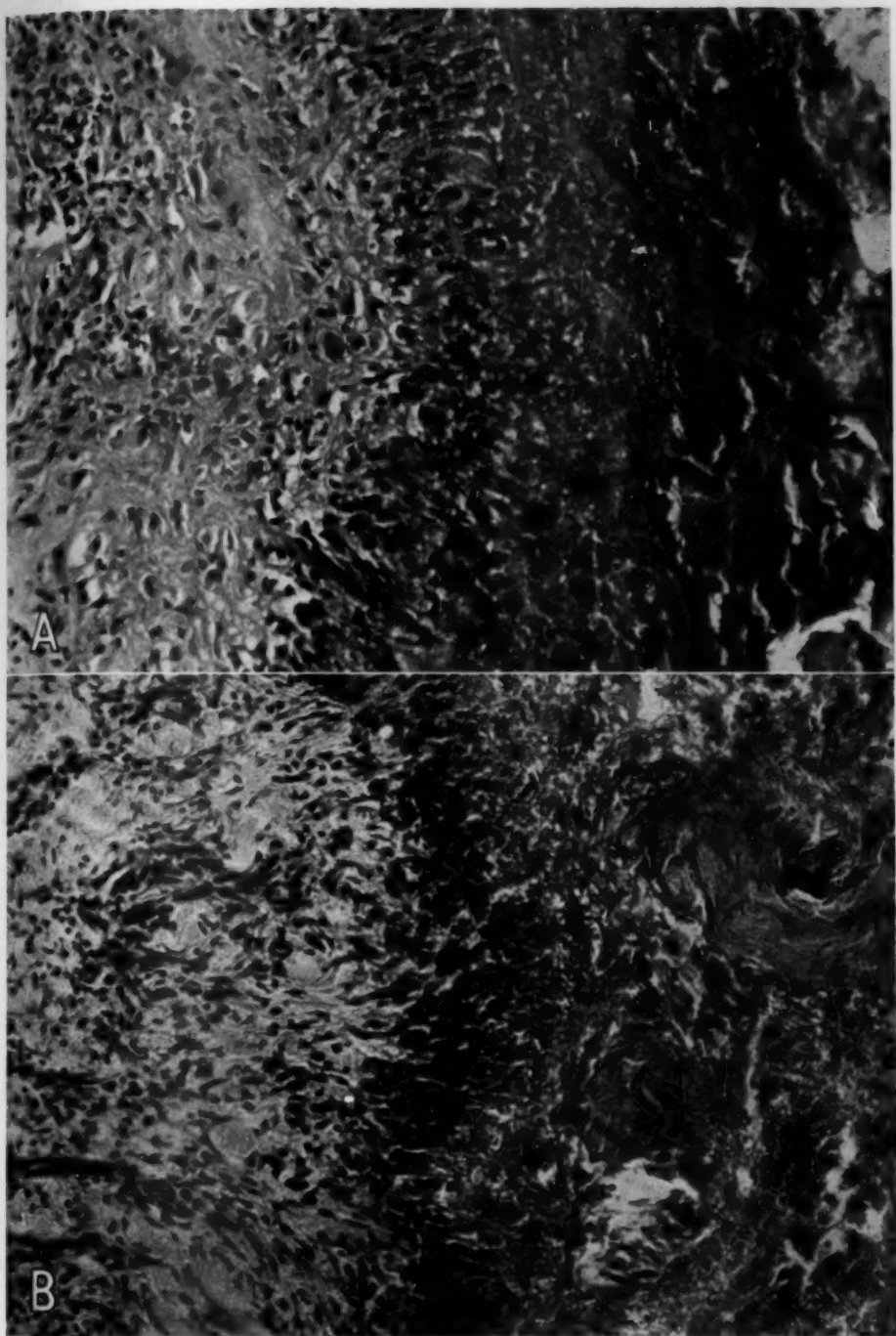


Fig. 5.—*A*, representative portion ($\times 187.5$) of a two month old subcutaneous rheumatoid nodule (specimen 777). Note the sharply outlined intermediate zone with palisaded cells and the complete necrosis and disintegration of tissue in the right half of the photograph.

B, necrotic material of a nodule in which a few remnants of connective tissue may be identified ($\times 187.5$). The changes are otherwise similar to those illustrated in *A*. This nodule (specimen 825) had been present in an arthritic patient for twenty months.

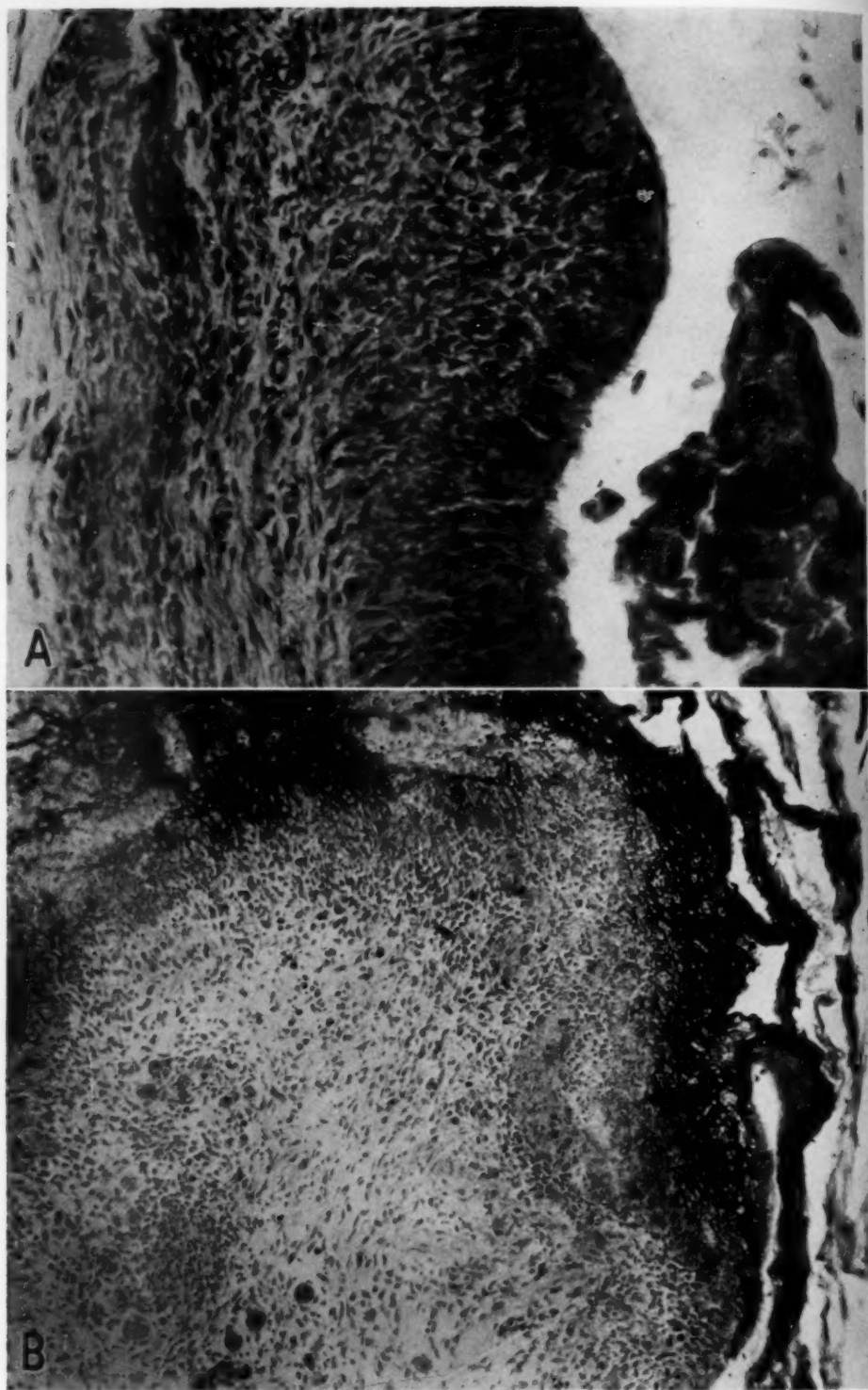


Fig. 6.—*A*, marked palisading of cells ($\times 236.5$) bounding a bursa-like cavity in the center of a subcutaneous nodule from an arthritic patient. The age of this nodule was unknown. The changes illustrated in this figure are not unlike those occasionally observed in the synovial tissue (fig. 7 *B*) and in the scleral nodules (scleromalacia perforans—fig. 7 *A*).

B, photomicrograph ($\times 113$) illustrating the fibrinous exudate near the surface of the granulation tissue that sometimes forms the boundary of the cavities in the subcutaneous nodules of patients with arthritis. This nodule (specimen 425) had been present for slightly more than six months.

necrosis. Some were detached and lying free in the central space. Fibrinous exudate was frequently seen on the inner surface of this zone and occasionally in the outer layer. Such exudative reactions simulated to a limited extent the primary exudation of the lesions of rheumatic fever. The intermediate zone of a well developed subcutaneous lesion of rheumatoid arthritis was strikingly like the scleral (fig. 7*A*) and synovial tissue nodules (fig. 7*B*) found occasionally in this disease.

The peripheral zones of the nodules of rheumatic fever and rheumatoid arthritis were not sufficiently characteristic to be of diagnostic significance. The encountered differences could usually be ascribed to difference in age of lesion or to variation in type of surrounding tissue. A diffuse infiltration of inflammatory cells was observed in both. Lymphocytes and mononuclear leukocytes predominated, but varying numbers of polymorphonuclear leukocytes, plasma cells and eosinophils were present. Moderate degrees of perivascular accumulation of lymphocytes were frequently seen in lesions of either type. Interstitial edema was more marked in rheumatic fever nodules. Vascular changes, varying from perivascular infiltration of inflammatory cells to diffuse inflammation and degeneration of the entire wall of the blood vessel were found frequently (fig. 8*A*). Thrombosis of such injured blood vessels was noted oftener in the nodules of rheumatoid arthritis (fig. 8*B*). This finding suggested that the central necrosis was due in part to infarction (fig. 8*B*). The vascular changes in the rheumatic fever lesions, except for the occasional thrombosis, were very similar to the widespread alterations of blood vessels described by Von Glahn and Pappenheimer.⁸ The peripheral zone of the rheumatic fever nodule was more vascular and exhibited greater degrees of hyperemia, perivascular exudation and swelling of the endothelial cells of the capillaries.

The foregoing microscopic observations have been interpreted as follows:

Inflammatory changes predominated in the rheumatic fever nodule and were associated with extensive exudation of plasma and cellular constituents of blood from injured capillaries and small vessels. The resulting diffuse interstitial edema and fibrin "soaking" of the interstitial tissues produced a prominent latticed arrangement of the swollen, acidophilic collagen bundles in the central area. Such lesions showed relatively little necrosis and degeneration. Scattered small foci of collagen necrosis and cellular proliferation resembling myocardial Aschoff bodies were frequently observed. Such lesions constituted one of the distinguishing features of the rheumatic fever nodule. They probably represented tissue responses similar to or identical with those occurring in the myocardium. All stages of repair were not seen. The

8. Von Glahn, W. C., and Pappenheimer, A. M.: *Am. J. Path.* 2:235, 1926.

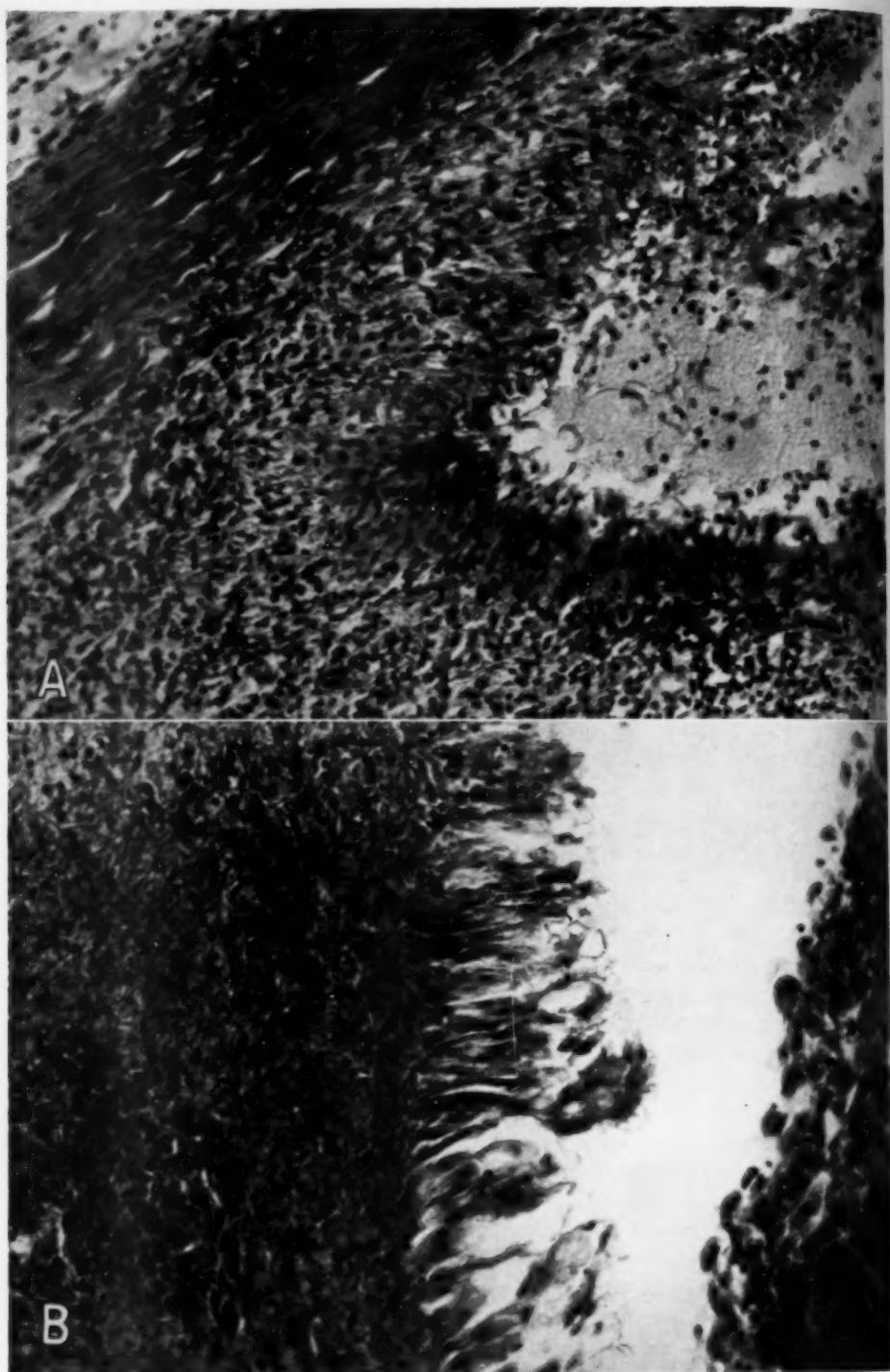


Fig. 7.—*A*, proliferative and inflammatory changes ($\times 226$) at the margin of a necrotic focus in the scleral nodule (scleromalacia perforans) of a patient with rheumatoid arthritis. A comparison of this lesion with a subcutaneous nodule from the same patient (specimen 476) or with other rheumatoid nodules revealed similar or identical features. Note the palisading bordering on the zone of necrosis and degeneration.

B, hypertrophy and hyperplasia of synovial lining cells with associated chronic inflammatory changes in subsynovial tissues ($\times 226$). Such changes occasionally result in lesions that are very similar to those observed in the subcutaneous nodule. This specimen of synovial membrane and a typical subcutaneous nodule (specimen 730) were obtained from a patient with rheumatoid arthritis.

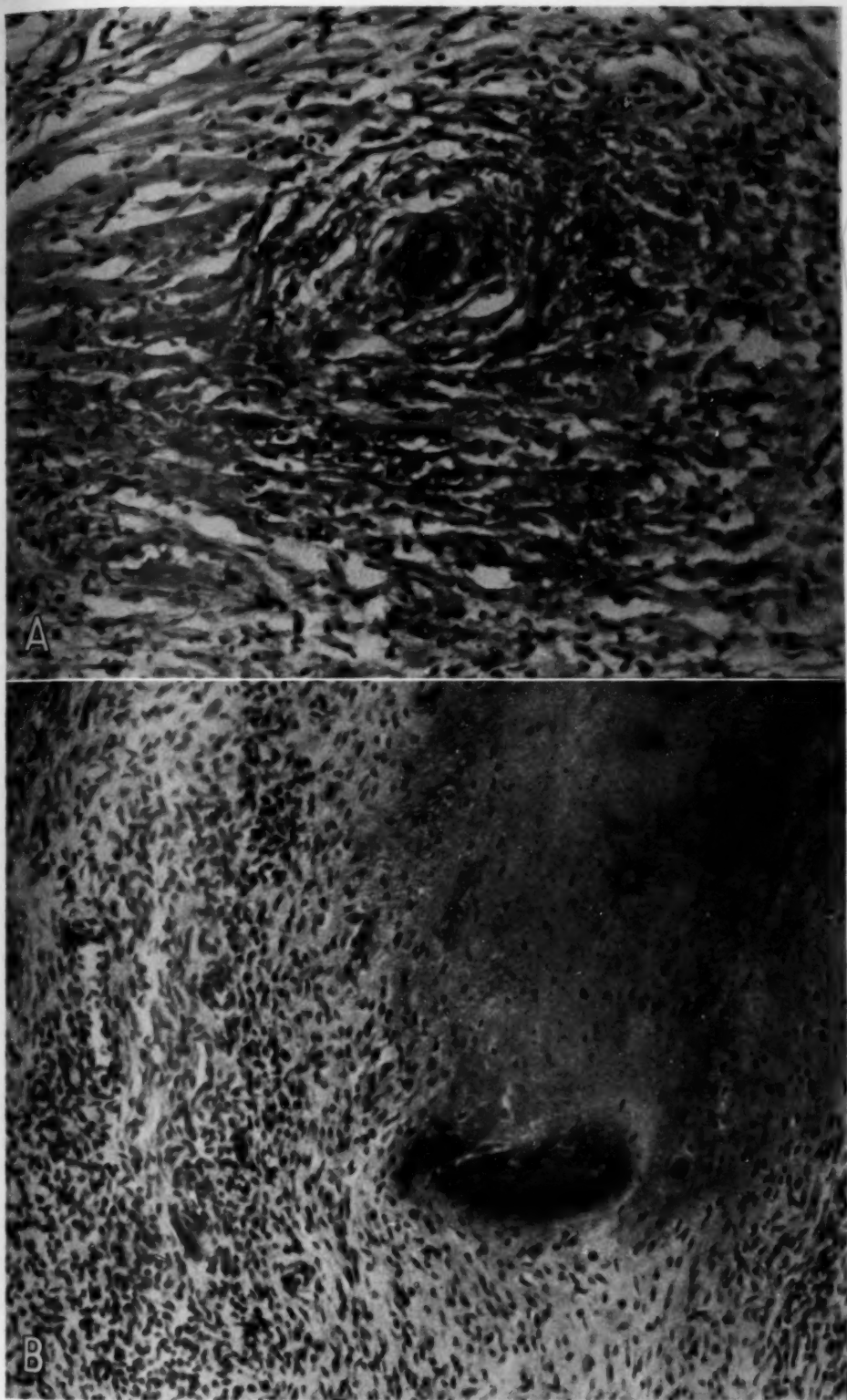


Fig. 8.—*A*, thrombosed blood vessel showing inflammatory changes in its wall. Such vessels were occasionally observed in the nodules of both rheumatoid arthritis and rheumatic fever. The arthritic nodule from which this photograph was made (specimen 835a) had been present for two and one-half years. Such marked vascular changes were rarely found in the rheumatic fever nodule.

B, photomicrograph ($\times 260$) illustrating the extensive tissue necrosis that sometimes occurred in conjunction with thrombosed blood vessels at the margins of well developed subcutaneous rheumatoid nodules.

changes that were observed suggest that repair takes place promptly, with only slight cicatrization, once the inflammatory exudate has been removed.

Necrosis of large areas of connective tissue and of all structures contained therein was the characterizing feature of the nodule of rheumatoid arthritis. Overgrowth of connective tissue and marked hyalinization of collagen appeared to be the primary changes in one lesion of short duration. This proliferative response appeared to have been followed by necrosis. The presence of thrombosed vessels in the younger nodules suggested that such lesions may have been initiated by infarction. Regardless of the mode of onset, the necrotic centers were sharply demarcated by rows of radially arranged cells showing evidence of rapid and long-continued proliferation. In well developed lesions the necrotic areas had undergone more complete disintegration, leading to liquefaction and subsequent cavity formation. These observations suggest that the initial development of such nodules is the result of a lethal injury to connective tissues by some unknown deleterious agent of strong potency. Injury of blood vessels with thrombus formation may be an important factor in some instances. It would appear that the responsible agent continues to act in the central necrotic focus causing the cells in the intermediate zone to continue to proliferate rapidly and to become palisaded in a manner designed to repair the injured tissue and perhaps to localize the injurious agent. Such a sequence of injury, necrosis and reparative proliferation apparently continues for months or years. Increasing amounts of connective tissue are formed at the periphery. In many instances a dense fibrous scar results. Occasionally the necrotic area undergoes calcification.

It will be seen from the table that the pathologic interpretation, although made without knowledge of the patient's disease, agreed in most instances with the final clinical diagnosis. The nodules from 2 of the 3 patients with rheumatoid arthritis who had heart disease that was probably of rheumatic origin showed the principal features of the lesions of rheumatoid arthritis. The third specimen, although less characteristic, was also considered to be of the same type. The nodule obtained from the child with probable rheumatoid arthritis presented the microscopic changes found in both types of subcutaneous lesions. The extensive necrosis of tissue, moderate palisading and cavity formation were more like those of the rheumatoid nodules.

One nodule (445) from a patient with typical rheumatoid arthritis exhibited the characteristic features of a lesion of rheumatic fever and was so diagnosed. Two specimens (2 and 175), although the alterations were not absolutely diagnostic, were interpreted as probably nodules of rheumatic fever. This was in accord with the clinical findings.

The most puzzling diagnostic problems were encountered in the case of a man of 56 years who had suffered from a moderately severe rheumatoid arthritis for three years. Countless subcutaneous nodules had been present for months over many of the bony prominences. Those removed post mortem (for example, 790) were comparable to the one (664) examined the year before. They all showed the changes which are considered diagnostic of rheumatoid arthritis. The pericardial and pleural lesions resembled the subcutaneous nodules of rheumatic fever. This case will be described in detail in a subsequent report.

COMMENT

One reason for undertaking this study was to determine whether or not the subcutaneous nodules of rheumatoid arthritis and rheumatic fever are disease specific. Similar nodules have not been observed in degenerative joint disease (hypertrophic or degenerative arthritis). Gross examination does not permit one to distinguish them from isolated nodular lesions, such as neurofibroma, fibroma, sclerosing hemangioma, ruptured sebaceous cyst or a localized area of traumatized fat or connective tissue. The same is also true with respect to the subcutaneous lesions observed in diabetes, gout, yaws, tuberculosis and syphilis. These various lesions, however, can usually be distinguished readily from the subcutaneous nodules of rheumatoid arthritis and rheumatic fever by microscopic examination once one becomes thoroughly acquainted with the evolutionary and healing phases of the latter.⁹ The juxta-articular nodules of syphilis have been thought by Hopkins¹⁰ to resemble more nearly those seen in the rheumatic diseases. Others¹¹ have stated that histologic differences are readily detected. Although we have never had the opportunity to study the juxta-articular nodules of syphilis, it should be mentioned that none of the patients in the present series showed clinical evidence of syphilis. One subject with rheumatic fever (specimen 833) had serologic evidence of syphilis, two of three Hinton tests being positive. Tests were not available on 2 patients with rheumatic fever and 4 patients with rheumatoid arthritis; the tests on all others were negative.

Although it has been possible in most instances to distinguish the nodule of rheumatoid arthritis from that of rheumatic fever, it is readily apparent that both present similar or identical structural and cytologic features. Differentiation is generally possible because one or more of the pathologic alterations usually predominate. The changes observed in the nodule of rheumatic fever have been interpreted as being due

9. Dawson.¹⁸ Collins.^{1h} McEwen.⁷

10. Hopkins, H. H.: *Bull. Johns Hopkins Hosp.* **49**:5, 1931.

11. Crouzon, O., and Bertrand, I.: *Bull. et mém. Soc. méd. d. hôp. de Paris* **50**:1401, 1926. Dawson.¹⁸ McEwen.^{4a}

chiefly to injury of small blood vessels with subsequent exudation of plasma and blood cellular constituents into the connective tissues. In addition, small focal lesions similar or identical to the myocardial Aschoff nodule are frequently seen. These features are different from those of the nodule of rheumatoid arthritis, in which marked proliferation and degeneration of the connective tissue predominate and Aschoff-like nodules are rarely if ever seen. None were observed in the 67 nodules of rheumatoid arthritis examined. Exudation is rarely a prominent feature of these lesions. Such an interpretation of the observed pathologic changes affords an explanation for the differences in time of appearance, duration and size of the nodules in these diseases. The nodule of rheumatic fever, being chiefly an exudative phenomenon, might be expected to appear more quickly and resolve more promptly, leaving little or no residuum. The continued proliferation and necrosis of the nodule of rheumatoid arthritis, on the other hand, would explain its longer duration and larger average size. The process of repair would require more complicated mechanisms, and hence cicatrization and calcification would occur much oftener.

The salient pathologic features were observed in both young and old nodules, indicating that age is not an important factor in the differentiation of such lesions. The same can be said for the age of the patient and the size of the nodules.

The distribution of the nodules was much the same in the two diseases and very similar to the findings of other workers.¹² The sites commonly affected were those subjected most frequently to trauma, suggesting that this is a predisposing factor in the production of these lesions.¹³

These morphologic observations do not permit conclusions concerning the nature of the agent or agents causing these nodules. Microorganisms were never observed in any of the appropriately stained sections. Cultural methods and animal inoculations likewise failed to disclose infection. Nevertheless, the observed pathologic changes could be the result of the activity of an infectious agent which to date has not been isolated. Further comparison of the various manifestations of tissue hypersensitivity with both types of nodules should be made. That such lesions might be caused by a chemical agent has been suggested by Verhoeff and King.¹⁴ They concluded that the type of reaction seen in the scleral nodules (scleromalacia perforans) closely

12. Findlay, L.: *The Rheumatic Infection in Childhood*, New York, William Wood & Company, 1932, p. 107. Dawson.¹⁵

13. Massell, B. F.; Mote, J. R., and Jones, T. D.: *J. Clin. Investigation* **16**: 125, 1937.

14. Verhoeff, F. H., and King, M. J.: *Arch. Ophth.* **20**:1013, 1938.

resembled that which follows the deposition of monosodium urate. This suggestion should be investigated.

The nodules of rheumatoid arthritis and rheumatic fever differ as much from one another as do the granulomas of syphilis and tuberculosis, suggesting that they may be due to different agents. The clinical and pathologic differences of rheumatoid arthritis and rheumatic fever also favor such an interpretation. It is therefore suggested that the readily accessible subcutaneous nodules should be studied by new and untried methods in the hope that more may be learned concerning their genesis and cause. The present study has yielded information that should be useful in differential diagnosis.

RATE OF DENTIN FORMATION IN INCISOR TEETH
OF GUINEA PIGS ON NORMAL AND ON
ASCORBIC ACID-DEFICIENT DIETS

PAUL E. BOYLE, D.M.D.

OTTO A. BESSEY, Ph.D.

AND

PERCY R. HOWE, D.D.S.

BOSTON

The thesis of Wolbach and Howe¹ that vitamin C (ascorbic acid) is essential to the normal formation of certain intercellular matrices was based in part on observations of changes in the cells and dentin matrix of the teeth of scorbutic guinea pigs. Their findings clearly demonstrated atrophy of the odontoblasts with decreased and irregular formation of an amorphous calcified dentin in the late stages of the deficiency. Höjer² and Wolbach³ stated impressions gained from histologic examination that a quantitative relation exists between the amount of vitamin C administered to guinea pigs and the quantity of intercellular material produced. Objective measurements by which such impressions may be confirmed can hardly be obtained from study of the matrices of white fibrous tissue, cartilage or bone, the tissues other than dentin characteristically affected by ascorbic acid deficiency. The dentin, unlike bone, which it resembles in many respects, is not subject to physiologic resorption and rebuilding and therefore is peculiarly suitable for such an investigation.

This paper is concerned with the normal rate of apposition of dentin in the incisor teeth of the guinea pig and with the effect of complete and of partial deficiency of ascorbic acid on this rate. The data presented are of value in establishing a quantitative relation between ascorbic acid intake and matrix formation, and are also important as a basis for future studies on the effects of various physiologic processes on dentin formation and on ascorbic acid metabolism.

From the Department of Operative Dentistry, Harvard Dental School, the Department of Pathology, Harvard Medical School, and the Forsyth Dental Infirmary.

The expenses of this investigation have been defrayed in large part by a grant from the Carnegie Corporation to the University Committee on Research in Dental Medicine.

1. Wolbach, S. B., and Howe, P. R.: *Arch. Path.* **1**:1, 1926.
2. Höjer, J. A.: *Acta pædiat.*, 1924, supp. 3, p. 8.
3. Wolbach, S. B.: *New England J. Med.* **215**:1158, 1936.

In the incisor teeth of guinea pigs the dentin is being continuously deposited at the formative end to compensate for the wearing away of tooth structure at the incisal end. The growth pattern is essentially the same as that of the incisor of the rat, which has been described by Schour and Steadman.⁴ The rate of eruption of the lower incisor teeth of guinea pigs as determined by Schadle and co-workers⁵ is about 2 mm. per week. At the formative end of the tooth circularly arranged tall columnar cells, the odontoblasts, become differentiated from the mass of embryonic-like connective tissue, and a matrix of collagenous material appears between their basal surfaces and the epithelial cells of the enamel organ with which they were previously in contact. They move incisally

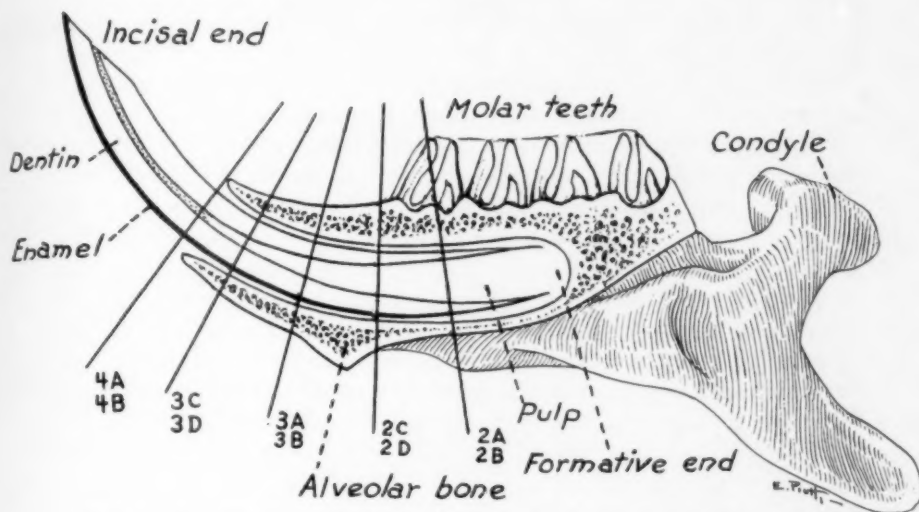


Fig. 1.—A drawing of the right half of the lower jaw of a guinea pig viewed from the lingual aspect. The overlying bone and the dentin to the center of the incisor tooth have been ground away. The enamel appears as a narrow black border on the convex labial side. The dentin increases in thickness from the proximal toward the incisal part of the tooth until the pulp chamber is obliterated at the level where the tooth emerges from the alveolar bone. The approximate planes at which the cross sections shown in figures 2 to 4 were taken are indicated by the transverse lines correspondingly numbered.

at approximately the rate of tooth eruption and at the same time retreat toward the center of the pulp, leaving more and more dentin matrix behind them. This matrix becomes calcified a short distance from the

4. Schour, I., and Steadman, S. R.: *Anat. Rec.* **63**:325, 1935.

5. Schadle, A. R.; Valvo, N. I., and Eckhert, K. M.: *Anat. Rec.* **71**:497, 1938.

ends of the odontoblasts. The intervening uncalcified tissue is called the predentin. Each odontoblast leaves in the dentin a cell process, which increases in length as the cell moves centrally. Because of their deposition of dentin in a unidirectional manner, the odontoblasts have been termed "polarized osteoblasts" by Wolbach and Howe.⁶ Since the peripheral layer of dentin is deposited first, the odontoblasts become crowded more and more closely together as they migrate incisally and centrally. As the tooth is about to emerge from the alveolar bone, the odontoblasts begin to degenerate, form dentin of an inferior quality and finally become incorporated in the calcified pulp tissues which seal off the pulp chamber. Their life cycle from differentiation at the formative end to degeneration at the incisal end occupies about five weeks (fig. 1).

The literature concerning the effect of ascorbic acid deficiency on the teeth of guinea pigs has been reviewed recently by Dalldorf.⁷ Atrophy and disorientation of the odontoblasts and deposition of dentin of inferior quality after a period of from seven to ten days on an ascorbic acid-free diet are characteristic findings. This dentin (osteodentin, pulp bone, calcific scar tissue) resembles the secondary dentin of human teeth. It may contain recognizable dentinal tubules, fewer and more tortuous than those in normally formed dentin, or it may incorporate atrophic cells and resemble bone. It somewhat resembles the last product of the senescent odontoblasts in the normal incisor but occurs in greater amounts and nearer the formative end of the tooth. In complete absence of ascorbic acid for four weeks the atrophy of odontoblasts and the deposition of secondary dentin extend back to the formative end of the incisor.⁸ In the teeth of guinea pigs given inadequate daily doses of ascorbic acid (0.3 mg.) for long periods of time the dentin at the formative end of the tooth always appears histologically normal, while slightly farther incisally dentin of inferior quality projects in long spicules toward the center of the pulp cavity.⁹ A method for the assay of vitamin C based on the extent of these histologic alterations in the tooth has been developed by Höjer¹⁰ and modified by Key and Elphick.¹¹ It is based on the observation that the degree of histologic change depends on the amount of ascorbic acid administered.

A means of marking dentin was reported by Marshall,¹² who described a vital staining of the incisor teeth of rats by alizarin and

6. Wolbach, S. B., and Howe, P. R.: *Am. J. Path.* **9**:275, 1933.

7. Dalldorf, G.: *J. A. M. A.* **111**:1376, 1938.

8. Fish, E. Q., and Harris, L. J.: *Phil. Tr. Roy. Soc., London*, s.B **223**:489, 1934.

9. Boyle, P. E.; Wolbach, S. B., and Bessey, O. A.: *J. Dent. Research* **15**:331, 1936.

10. Höjer, J. A.: *Brit. J. Exper. Path.* **7**:356, 1926.

11. Key, K. M., and Elphick, G. K.: *Biochem. J.* **25**:888, 1931.

12. Marshall, J. S.: *J. Dent. Research* **3**:241, 1921.

other dyes. By giving injections at known intervals of time, he estimated the rate of apposition of dentin to be 0.01 mm. per day. Schour and co-workers¹³ published a preliminary report on the rate of dentin formation as measured by alizarin in the teeth of rats on diets deficient in vitamin A. Ziskin and Applebaum¹⁴ used alizarin to determine the rate of dentin apposition in the teeth of hypophysectomized monkeys.

In making the measurements and observations to be described in this report we have similarly used periodic injections of sodium alizarin sulfonate as a means of marking the dentin of the continuously growing incisors of the guinea pig.

PROCEDURE

Periodic injections of alizarin were given to guinea pigs during the time they were being adjusted to various intakes of ascorbic acid and after they had become adjusted. Each injection of alizarin stained the dentin being formed at that particular time throughout the length of the tooth. A few days after the last alizarin was given the animals were killed, serial sections of the incisor teeth prepared and the bands of dentin laid down between alizarin lines measured. There was obtained from these data a complete quantitative picture of the rate of dentin formation in every part of the tooth under varying conditions of ascorbic acid metabolism.

Observations from a number of preliminary experiments served as a guide in the selection of those details of procedure, such as dosage and intervals of time, most suitable for the purpose.

MATERIAL AND METHODS

Female guinea pigs, 144 in all, 6 to 8 weeks old and weighing approximately 300 Gm., were used. One week previous to the start of the experimental procedure they were placed on a vitamin C-free basal diet and given small amounts of greens. The composition of the diet was as follows:

	Per Cent
Soybean meal ¹⁵	36
Ground rolled oats.....	25
Skimmed milk powder ¹⁶	20
Brewers' yeast.....	4
Alfalfa meal ¹⁶	8
Peanut oil.....	5
Calcium carbonate (CaCO ₃).....	1
Sodium chloride (NaCl).....	1
1 cc. of cod liver oil, given by pipet twice weekly.	

13. Schour, I.; Smith, M. C., and Hoffman, M. M.: *Proc. Soc. Exper. Biol. & Med.* **39**:447, 1938.

14. Ziskin, D. E., and Applebaum, E.: *J. Dent. Research* **18**:287, 1939.

15. The meal was autoclaved under 15 pounds' (7 Kg.) pressure for one hour.

16. This was heated in an oven (100 C.) in shallow pans with frequent turning for eight hours.

EXPLANATION OF FIGURES 2 TO 4

In figures 2 to 4 the sections shown at the left hand are ground cross sections of the lower incisor teeth of a guinea pig given greens daily for seventeen days (positive control); the sections shown at the right hand are corresponding sections from a guinea pig given no supplement for seven days and 0.25 mg. of ascorbic acid for ten days. Sections *A* and *B* in figure 2 were cut near the basal ends of the incisors; the plane of section is shown in figure 1 by the transverse line labeled to correspond (2 *A*-2 *B*). Each succeeding pair of sections were cut nearer the incisal parts of the teeth at planes shown by the transverse lines in figure 1 labeled 2 *C*-2 *D*; 3 *A*-3 *B*; 3 *C*-3 *D* and 4 *A*-4 *B*.

Each photomicrograph is mounted with the enamel-covered side of the tooth down and the lateral side to the left. The junction between enamel and dentin is labeled *DEJ*. The sections are unstained except by intravital injections of alizarin made seventeen, ten and seven days prior to killing the animals, indicated by lines 1, 2 and 3 in the figures.

The dentin between line 3 and the pulp was formed during the final seven days of the experiment. It should be compared in each series as well as in each pair of figures.

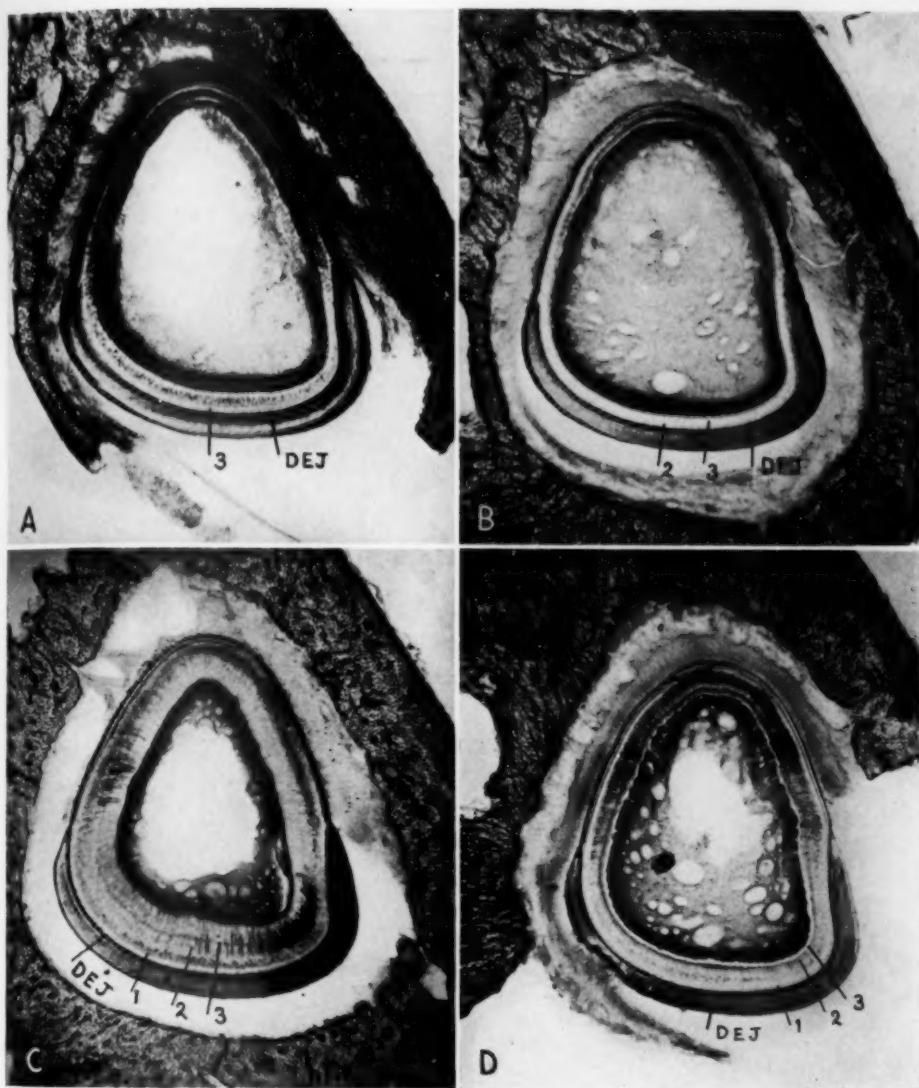


Fig. 2.—*A* shows only line 3, made by the injection seven days previously; $\times 25$. The dentin internal to line 3 is histologically normal. A detail drawing of a similar section is shown in figure 5 *A*. *B* shows lines 3 and 2, indicating that the plane of section is farther from the formative end of the tooth than *A*; $\times 25$. The dentin internal to line 3 is histologically normal but less than half as wide as in *A*. A detail drawing of a similar section is shown in figure 5 *B*. In *C* the band of dentin internal to line 3 is wider than in *A*; $\times 25$. In *D* the band of dentin internal to line 3 is fairly uniform on the enamel-covered part but much narrower than in *C*; $\times 25$. The dentin produced in the final seven days in the cementum-covered part of the incisor shows spicules projecting toward the center of the pulp.

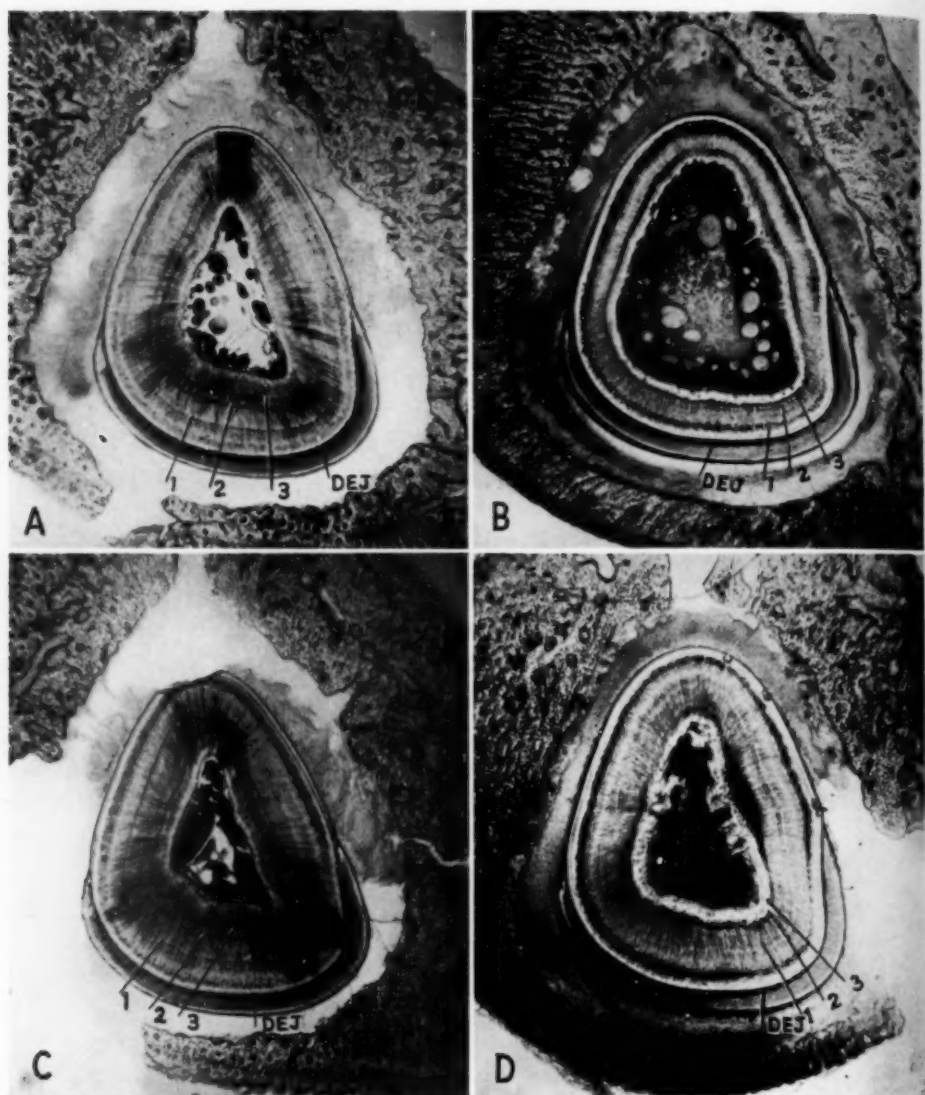


Fig. 3.—In *A* the dentin band internal to line 3 is wider than in *A* and *C* of figure 2, especially toward the lateral (left) side. In *B* the dentin band internal to line 3 is qualitatively as well as quantitatively subnormal. *C* shows the increasing variation in width of the dentin band internal to line 3 in different parts of the same cross section. *D* shows the qualitative and quantitative changes in dentin formed in the final seven day period more marked than in the preceding sections shown in these figures.

That the diet is complete in all respects other than vitamin C is shown by the fact that on it with the addition of ascorbic acid young guinea pigs have grown vigorously and have remained in normal condition for as long as a year.

The tests were started by withdrawing the greens and twenty-four hours later giving 15 mg. of sodium alizarin sulfonate¹⁷ intraperitoneally. After seven days, another 15 mg. of alizarin was given, and the administration of graded daily doses of ascorbic acid was begun. Preliminary experiments indicated that qualitatively normal dentin was deposited for at least seven days and not more than ten days if no ascorbic acid was given. After three days, when the animals had been allowed to become adjusted to their particular ascorbic acid intake, another 15 mg. of alizarin was given. Positive control groups of animals were given greens throughout the experimental period, and negative controls were treated as

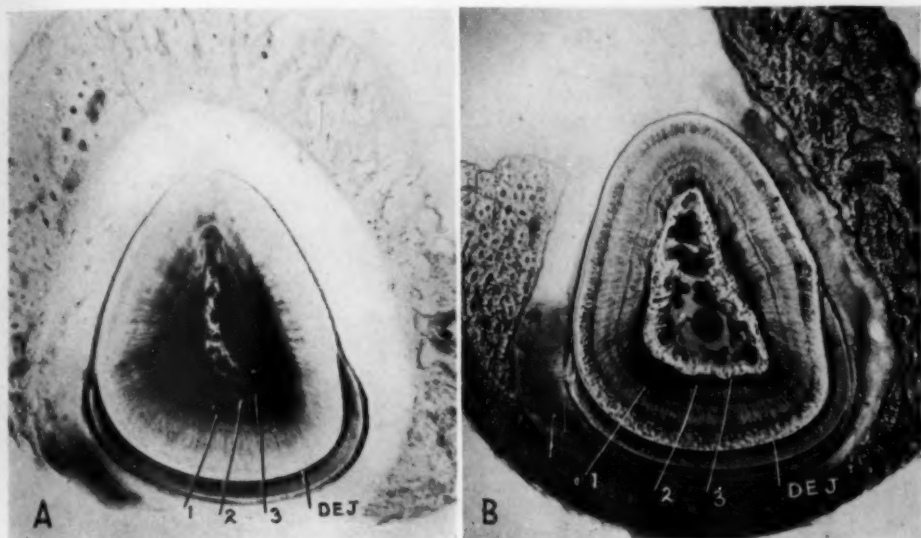


Fig. 4.—*A* shows the alizarin lines becoming blurred as the odontoblasts cease depositing dentin to become incorporated in the calcified tissue in the central part of this cross section. In *B* it may be seen that the dentin internal to line 3 is very irregularly formed and resembles the calcified tissue in the center of *A*.

17. A 5 per cent solution was prepared by dissolving 5 Gm. of alizarin red S (alizarin monosodium sulfonate), a certified biologic stain, in 85 cc. of hot water. To the cooled solution 14 cc. of 0.1 sodium hydroxide was slowly added to bring the p_H near the neutral point. Staining of the boundary between the uncalcified predentin and the calcifying dentin occurs during the few hours subsequent to the injection of alizarin, before the concentration of the dye in the blood has been reduced by renal excretion. Staining depends on the formation of deep red insoluble calcium salts. Although the peripheral zones of most calcified structures (e.g., bone) are stained to varying degrees, the actively calcifying region of the dentin is particularly heavily stained, owing to its abundant blood supply and to the accessibility of the newly formed calcium salts to contact with the dye.

described for the experimental group except that the daily dose of ascorbic acid was omitted throughout the experiment.

At the end of the seventh day following the last injection of alizarin the animals were chloroformed. The heads were severed from the bodies and, after removal of most of the skin and muscle, fixed in neutral solution of formaldehyde U. S. P. diluted 1:10. Following fixation for twenty-four hours or longer, the lower jaw was disarticulated and the right and left halves separated. Serial cross sections approximately 1 mm. in thickness were sawed from the formative toward the incisal ends of both incisor teeth of a number of animals, using a high speed dental engine and a "lightning disk." Selected sections from all animals were ground and polished, dehydrated and mounted in gum dammar.¹⁸

Measurements of the width of the bands of dentin outlined by the alizarin lines were made with a micrometer eye piece and recorded to the nearest micron. Measurements were made along the dentinal tubules in the midportion of the enamel-covered part of the tooth. Cross sections taken near the formative end of the tooth (level of the transverse line 2A-2B in fig.1) include only the line made by the injection of alizarin seven days previously (fig. 2A, line 3). Those taken farther incisally include the lines made by the injection of alizarin seven, ten and seventeen days previously (figs. 2C to 4B, lines 3, 2, 1). The distance of the alizarin lines from the outer periphery of the dentin increases as cross sections farther from the formative end of the tooth are examined (figs. 2 to 4). We did not attempt to keep the cross sections of the incisor teeth in serial order but used the distance from the dentoenamel junction to alizarin line 3 as an index of the relative position of the section examined. The distance in microns from the dentoenamel junction to line 3 was labeled D E for convenience. The D E of each cross section examined was recorded together with the width of the band of dentin formed in the final seven days of the experiment.¹⁹

A study of the diagrammatic longitudinal section in figure 6 may serve to clarify the use of the distance D E as an index of the plane of cross section. In this figure the line made by the injection of alizarin seven days previously is labeled 3. The dentin above line 3 has been formed during the final seven days of the experimental period. An odontoblast differentiating from the embryonic-like connective tissue at the formative end of the tooth is diagrammed at A. Another at B has been depositing dentin for seven days, during which time it has migrated anteriorly approximately 200 microns and receded pulpally about 95 microns. At C, D and E are shown odontoblasts which have been in

18. In some instances longitudinal sections of the opposite incisor tooth were made. Considerable difficulty was encountered in preparing specimens which included the entire length of the incisor teeth in midsagittal sections, but several satisfactory for microscopic examination were secured. Occasional sections were stained in alizarin solution or in silver nitrate solution.

19. In a ground section the boundary between the predentin and the calcified dentin appeared as a dark band, and it was considered as marking the inner deposit of dentin formed in the final seven days of the experiment. Its width is about the same as that of the alizarin lines, and it is this band of calcifying dentin which is colored by intra vitam or in vitro alizarin staining.

normal function for fourteen, twenty-one and twenty-eight days, respectively. A cross section cut at the level of the odontoblast at *B* would have a D E of 0; from one cut at *C*, about 95 microns (detail drawing, fig. 5 *A*); one cut at *D*, 190 microns and one cut at *E*, about 225 microns. Thus it may be seen that the D E to line 3 in a cross section increases as sections are taken farther from the formative end of the tooth, and if the D E is known the plane of section can readily be determined.

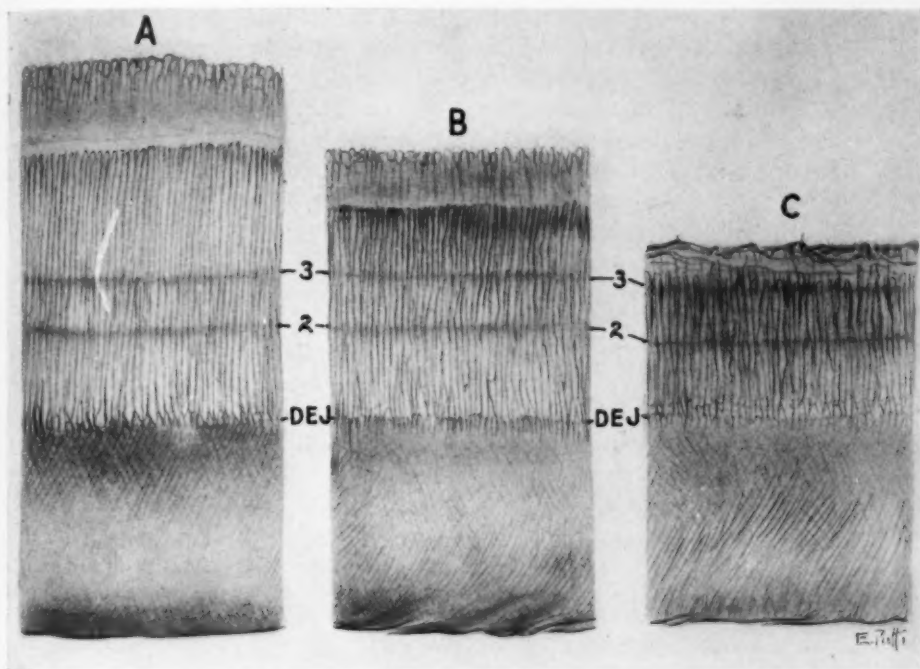


Fig. 5.—Drawings illustrating the detail of tooth formation in cross sections taken at the level of odontoblast *C* in figure 6. Lines 3 and 2 were produced by injection of alizarin seven and ten days previously. DEJ is the dentoenamel junction. The bands of dentin pulpal to (above) line 3 should be compared. *A* represents a positive control (adequate ascorbic acid). *B* represents a guinea pig given an inadequate amount of ascorbic acid, 0.25 mg. daily, for ten days. The dentin formation in the final seven days is quantitatively reduced as compared with the normal. *C* represents a negative control (no ascorbic acid for seventeen days). The dentin formation in the final seven days is quantitatively and qualitatively subnormal. Approximate magnification, $\times 200$.

The lines made by injections of alizarin ten and seventeen days previously are labeled 2 and 1, respectively, in figure 6. These lines mark the internal border of the dentin at the time the particular injections of

alizarin were made. If moved apically they could be superimposed on each other and on the internal border of the dentin. Therefore in cross sections showing three alizarin lines the distance from the dentoenamel junction to any line may be used to determine the position of the section examined at the time the injection of alizarin was given. Thus the use of three alizarin lines made it possible to determine the rate of dentin formation in the positive control animals in the same relative position in the same tooth at successive periods of time. In the group of animals given no ascorbic acid (negative controls) lines 1 and 2 served to determine the period of time during which histologically normal dentin continued to be deposited, which is an index of the time of depletion of the body stores of vitamin C. In the groups of animals given ascorbic acid lines 1 and 2 demarcated the dentin formed during the periods of depletion and adjustment to the supplement. Line 3 in all groups

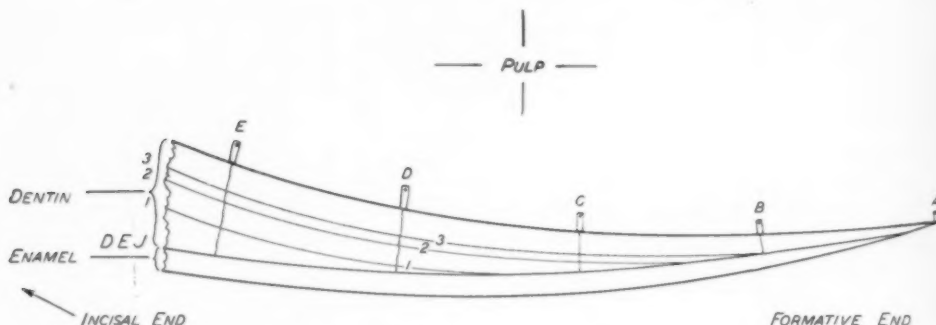


Fig. 6.—A detail drawing of the enamel-covered part of the formative end of the normal incisor in longitudinal section. An odontoblast beginning to deposit dentin is labeled *A*. At *B* an odontoblast which has been depositing dentin for seven days is drawn. It has migrated incisally from the position *A* in this period and has receded pulpally leaving its process in the dentin. At *C*, *D* and *E* odontoblasts which have been depositing dentin for fourteen, twenty-one and twenty-eight days are shown. Line 3 is the line made by injection of alizarin seven days previously, and the dentin between this line and the odontoblasts has been deposited during the last seven days. Line 2 represents an injection of alizarin ten days previously and line 1 an injection seventeen days previously. All lines would coincide with the inner dentin border if moved apically to start at odontoblast *A*. A cross section taken at *B* would show only line 3; one at *C*, lines 2 and 3 (see fig. 5 *A*), and sections at *D* and *E*, all three lines (see figs. 2 *C*, 3 *A* and 3 *C*). Approximate magnification, horizontally, $\times 12.5$, vertically, $\times 25$.

marked the periphery of the band of dentin formed in the final seven days of the experiment. Measurements of the width of this band were used to determine the relation between rate of dentin formation and intake of ascorbic acid.

OBSERVATIONS

Findings are described for (1) animals given adequate amounts of ascorbic acid throughout the experimental period (positive controls), (2) those given the basal ascorbic acid-free diet only (negative controls) and (3) those given graded supplements of ascorbic acid after a seven day depletion period.

Animals Given Adequate Amounts of Ascorbic Acid.—This group was composed of animals given the basal diet supplemented by greens throughout the experimental period. The rate of dentin apposition was found to be constant for a short distance at the formative end of the tooth (D E, 0-100), after which the rate rapidly increased to a maximum, which was maintained for a considerable distance. Near the incisal part of the pulp chamber the rate began to decrease and at the level where the tooth emerges from the alveolar bone dentin deposition ceased and was followed by the formation of a calcified pulp tissue. These findings are illustrated in figures 2 A, 2 C, 3 A, 3 C and 4 A, which are photomicrographs of a series of sections made from the teeth of one animal. The approximate levels at which the sections were taken are shown in figure 1. The transverse lines in this drawing are numbered to correspond with the numbers of the photomicrographs.

Variations in the distance between alizarin lines in different regions of the same cross section may be observed in all figures. However, in figures 2 A, 2 C and 3 A the bands of dentin bounded by alizarin lines are seen to be uniform in width in a particular region of a given cross section, e. g., the midportion of the enamel-covered side. Farther from the formative end of the tooth (fig. 3 C) the pulpal surface of the dentin becomes scalloped and irregular and in figure 4 A dentin formation ceases and is succeeded by the deposition of calcified pulp tissue. Figure 3 A shows the lines made by the alizarin injections particularly well, and a detailed description of the steps taken in recording data from this section may serve to illustrate the method followed for all sections. Figure 3 A, as do all the other figures, shows the enamel side of the tooth down, the lateral side to the left and the medial side to the right. The junction between the enamel and dentin is marked *DEJ*. The radially arranged tubules of the dentin converge toward the pulp. The concentric dark (red) bands made by injections seventeen, ten and seven days prior to killing the animal are numbered 1, 2 and 3, as in figure 6. Seventeen days previously the pulp tissue occupied the space inside line 1. During the next seven days the cells of the pulp formed new dentin and receded centrally to the position marked by line 2, in the next three days to line 3 and in the last week to the position shown in the photograph. The distance between lines 1 and 2 in the mid-enamel part of the section averages 120 microns, and the distance from the dentoenamel junction (*DEJ*) to line 1 is 155 microns (D E of line 1). The distance between lines 2 and 3 is 57 microns for three days, which

is at the rate of 133 microns for seven days. The distance between line 3 and the calcified dentin bordering the pulp tissue is 138 microns and the D E of line 3 is 332 microns.

These measurements were charted on graph paper with the D E in microns as the abscissa and the distance between alizarin lines as the ordinate. The measurements for all other sections of the incisors of the same animal were then plotted. Charts for each animal were prepared and an average curve drawn. A composite curve from the charts of all animals on greens was found to correspond closely with the composite curve from animals given supplements of 5.0 mg. of ascorbic acid per

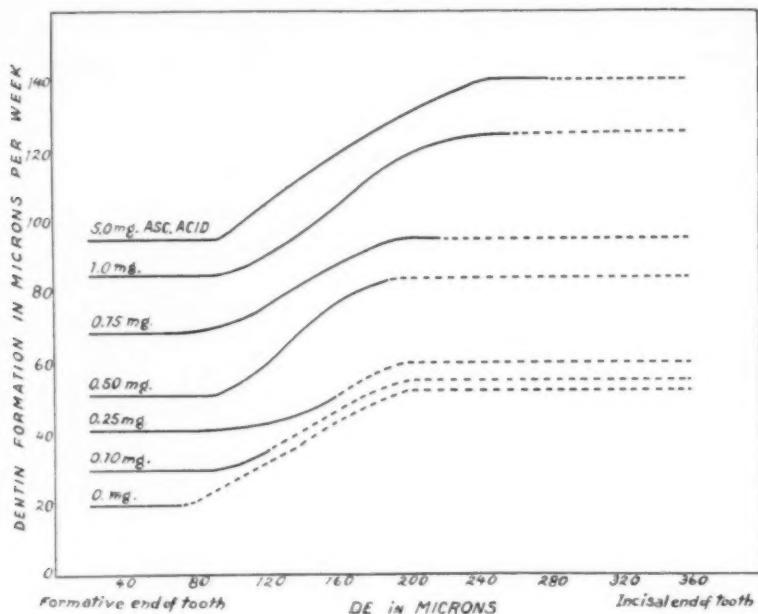


Fig. 7.—A summarization in graphic form of the rates of dentin formation at various levels of the guinea pig incisor for varying ascorbic acid intakes. Each curve represents average measurements of a large number of serial sections from a group of animals, all of which received ascorbic acid daily in the amount indicated by the figure at the beginning of the curve. The abscissas, 0-360 microns, represent relative distances (D E) of the cross sections from the formative end of the tooth. The ordinates, 0-140 microns, represent widths of the band of dentin formed in a week.

day. The latter curve is reproduced in figure 7. When measurements of sections from the same incisor, from opposite incisors of the same animal or from the incisors of different animals are compared, the D E's being approximately equal and less than 300 microns, a very satisfactory degree of uniformity is found. As may be seen from figure 7, the rate of dentin formation in the positive control and 5.0 mg. animals is rela-

tively constant for a D E of 0 to 100, then increases rapidly for D E's of 100 to 250. Sections taken farther incisally, D E greater than 250, show an increasing variation in the amounts of dentin formed by adjacent groups of odontoblasts (dotted line, fig. 7). The maximum rate of dentin apposition in the normal animal is about 180 microns per week. In the region shown in figure 4A where the D E reaches its maximum of between 500 and 600 microns, the alizarin lines become blurred and difficult to identify. There can be little doubt, however, that the rate of dentin formation is declining in this part of the tooth.

Ascorbic Acid-Free Diet Only (Negative Controls).—During the first week that the animals were on the basal diet, dentin which appeared histologically normal was deposited in all parts of the tooth, but the rate of deposition was slightly reduced. The band of dentin formed during the first seven days is bounded by alizarin lines 1 and 2. The average width of the band was 80 microns, compared with 95 microns for the positive controls (D E in both instances less than 100). During the succeeding three days the deposition of dentin with regularly arranged parallel tubules ceased abruptly (dentin bounded by lines 2 and 3). In some animals this apparently occurred on the eighth day; in others, on the ninth or tenth days. The dentin deposited between the tenth and seventeenth days, from line 3 to the pulp, was irregular, contained few tubules and resembled the dentin found in normal animals in the region where the pulp chamber becomes obliterated. Spikes of this irregular dentin projecting toward the center of the pulp chamber were found in cross sections taken at a distance from the formative end of the incisors of many of these animals. The dentin in sections with a D E (line 3) of less than 100 did not form spikes but was scalloped and irregular and the predentin was absent, the dentin being calcified to the ends of the atrophic odontoblasts (fig. 5C). It was difficult to measure accurately the width of the band of dentin deposited in the final seven days, even near the formative end of the tooth, because of this irregularity. However, the average of a number of sections from several animals was 20 microns in the region where animals on the normal diet averaged 95 microns (fig. 7).

Animals Given Supplements of Ascorbic Acid.—The supplements given were 5.0, 2.0, 1.0, 0.75, 0.5, 0.25 and 0.1 mg. of ascorbic acid daily. Solutions were freshly made in water distilled from glass apparatus and were given by mouth from graduated syringes. As mentioned in the previous section, during the seven day depletion period dentin containing regularly arranged parallel tubules continues to be formed, although at a slightly reduced rate. Animals receiving 5.0 mg. per day continued to form histologically normal dentin throughout the experiment in all parts of the tooth. The dentin deposited during the final seven days, bounded peripherally by line 3, was deposited at the same

rate as in the positive control animals. In animals receiving 2.0 and 1.0 mg. doses the dentin deposited was histologically normal, but the rate of formation was slightly less than normal (figs. 7 and 8).

Animals receiving 0.75 mg. or less showed striking histologic alterations in the dentin deposited in the final seven days, in all parts of the tooth except near the formative end. This abnormal dentin was very irregularly formed and projected in long spikes toward the center of the pulp (figs. 3 *D* and 4 *B*). The animals receiving 0.75 and 0.5 mg. usually showed more extensive spike formation than those receiving the lower doses, while the latter showed irregular dentin deposition nearer the formative end of the tooth. Those on 0.1 mg. resembled the negative control group in that the uncalcified predentin disappeared. The dentin in sections taken near the formative end of the tooth (*D E* of line 3 less than 80) showed the formation of dentin with regularly arranged parallel tubules in all groups, and the rates of dentin deposition in this region varied directly with the amounts of ascorbic acid given as shown in figure 7.

Figures 2 *B* and *D*, 3 *B* and *D* and 4 *B*, showing sections of an incisor of an animal on 0.25 mg. doses, illustrate the characteristic features of groups receiving inadequate supplements of ascorbic acid. These sections were taken at approximately the same planes as the sections shown in figures 2 *A* and *C*, 3 *A* and *C* and 4 *A*. Section *B* in figure 2 was taken slightly farther from the formative end of the tooth than section *A*. It shows dentin with parallel tubules. The predentin is very narrow. The distance from the inner alizarin line, 3, to the predentin is 41 microns, compared with 98 microns in section *A*. In section *D* in figure 2 the dentin on the enamel-covered side is still uniform in width. In other parts of the section spikes of irregular secondary dentin have appeared. In the succeeding figures the irregularity of the dentin formed in the final seven day period may be seen to become more marked. The width of this secondary dentin may in some areas equal or exceed the width of the band of dentin formed under normal conditions. This rapid deposition of dentin of inferior quality appears to be a compensating mechanism. Figure 4 *B* shows the central part of the tooth nearly filled with secondary dentin. Slightly farther incisally, calcified pulp tissue enclosing the remaining soft tissues and blood vessels would be found.

The effect of the intake of ascorbic acid on the formation of dentin is well shown in the drawings reproduced in figure 5. They are segments on the midenamel side of cross sections similar to those shown in figures 2 *A* and *B*. The level of section is approximately that of the odontoblast at *C* in figure 6. The enamel is in the lower part of each drawing, and the junction between enamel and dentin is marked *DEJ*. The lines made by injections of alizarin ten and seven days previously are labeled 2 and 3. The drawing *A* illustrates dentin formation in a

positive control animal. The dentin pulpal to line 3 contains regularly arranged parallel tubules. The predentin is represented as a clear area in which the tubules, although present, are seen with difficulty, and the odontoblasts are tall columnar cells bordering the predentin. The distance along the dentinal tubules from line 3 to the boundary between the calcified dentin and the predentin was 95 microns and the D E of line 3 was also 95 microns.

The drawing *B* in figure 5 shows a similar area from the tooth of an animal given 0.25 mg. supplements of ascorbic acid for ten days after a seven day period on the basal diet alone. The D E of line 3 is 95 microns. The formation of regularly arranged dentin was continued

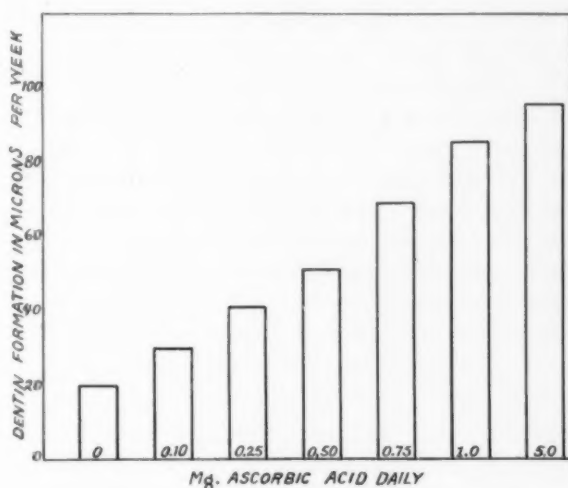


Fig. 8.—The graph shows that the amount of dentin produced during a given time at the formative end of the guinea pig incisor (D E, 0-100 microns) is proportional to the amount of ascorbic acid administered. The columns (0 to 100) represent the numbers of microns of dentin formed per week at various dosages of ascorbic acid. The number at the base of each column represents the milligrams of ascorbic acid administered daily.

from line 3 to the boundary between dentin and predentin and the distance averaged 41 microns, compared with 95 in the normal animal. The reduced height of the odontoblasts and the narrow border of predentin may be noted in the drawing.

The drawing *C* in figure 5 is of the tooth of a negative control animal. The formation of dentin with regularly arranged parallel tubules ceased at about the tenth day after deprivation of ascorbic acid. The irregular calcified dentin extends to the ends of the atrophic odontoblasts (not drawn). The amount of dentin formed during the final seven days was less than 20 microns in width.

Figure 8 summarizes the findings concerning the effect of graded amounts of ascorbic acid on the rate of deposition of dentin in a definite part of the tooth near the formative end (D E of line 3 less than 100). The rate of animals given 5.0 mg. of ascorbic acid coincides with that of animals given greens and is regarded as normal. Animals given decreasing doses show a proportional decrease in the rate of dentin deposition. The data on which figures 7 and 8 are based will be given in statistical form in a forthcoming publication describing an objective biologic assay method for the quantitative determination of vitamin C (ascorbic acid).

COMMENT

In animals on a normal diet the rate of the apposition of dentin as determined by injections of alizarin varies nearly 100 per cent in different parts of the same incisor tooth. Nevertheless, the rate is essentially the same in corresponding parts of the two incisor teeth of the same animal or of different animals, indicating a definite gradient of rate of growth.²⁰

These findings emphasize the necessity of establishing accurately the location in the tooth from which sections are taken before measurements of rate of dentin deposition can be treated in a quantitative manner. The use of the D E value of alizarin lines as described in this paper is a practical and accurate means to this end.

In animals receiving inadequate amounts of ascorbic acid the odontoblasts appear to differentiate normally and for a limited time, depending on the intake, continue to produce histologically normal dentin as they migrate incisally. That these bands of dentin may nevertheless be quantitatively reduced was previously inferred by the observation that the enamel-dentin ratio in the formative end of the tooth increases as the dose of ascorbic acid decreases.²¹ The present report establishes definitely that the apparently normal dentin near the formative end of the tooth is reduced in amount in ascorbic acid deficiency and, furthermore, that a direct relation exists between the quantity of dentin found in this region and the amount of ascorbic acid administered.

The occurrence of histologically normal dentin near the formative ends of the incisor teeth of animals given inadequate daily supplements of ascorbic acid while the dentin farther toward the incisal end is extremely irregular has been noted even in animals kept for as long as a year (unpublished data). During this period the teeth are completely renewed several times.

20. Thompson, D. W.: *On Growth and Form*, London, Cambridge University Press, 1917. Schour, I., and Poncher, H. G.: *Am. J. Dis. Child.* **54**:757, 1937.

21. Boyle, P. E.: *Am. J. Path.* **14**:843, 1938.

The formation of normal dentin for seven to ten days after an ascorbic acid-free diet is given coincides with the period during which stores of available ascorbic acid are being depleted. The material formed subsequently resembles the last product of the senescent odontoblasts and the calcified pulp tissue formed in the center of the incisor of the normal animal.

Undoubtedly a measurement of the total area of dentin produced during a given interval of time would be a more direct index of the quantity of dentin produced than the linear measurement used here. However, because the former method introduces several practical difficulties, we have measured the width of the bands instead of their areas.

SUMMARY

The use of alizarin to stain vitally the dentin formed during a particular period has made it possible to measure the marked variations in rate of deposition that occur in different parts of the incisor teeth of animals on adequate diets and to compare them with the rates found in corresponding parts of the teeth of animals on restricted amounts of ascorbic acid. The rate of dentin formation in definite areas near the formative end of the tooth has been found to be uniform in animals given the same supplements of ascorbic acid. After a standard depletion period the amount of dentin deposited in this region has been shown to vary directly with the amount of ascorbic acid administered. The findings demonstrate a measurable quantitative relation between the rates of formation of an intercellular substance, the dentin, and the amount of ascorbic acid administered to guinea pigs. They make possible the development of an objective biologic assay method for the determination of vitamin C.

OSTEOPOROSIS ASSOCIATED WITH EXTENSIVE METASTATIC CALCIFICATION AND CHRONIC RENAL DISEASE

CHARLES L. BROWN, M.D.

AND

I. W. GINSBURG, M.D.

PHILADELPHIA

Metastatic calcification is not uncommonly seen as a feature in conditions characterized by excessive destruction of bone. It has been observed in myelogenous leukemia, multiple myeloma and sarcomatous and carcinomatous metastasis to bone. It is sometimes associated with osteomyelitis and has been noted in parathyroid disease, both in adenoma and in idiopathic hyperplasia. It is a common finding in forms of long-standing renal insufficiency,¹ namely, renal hyperparathyroidism, renal osteitis fibrosa cystica and renal rickets. Castleman and Mallory² and Albright, Drake and Sulkowitch^{1a} stressed the histologic changes found in the parathyroid glands in renal hyperparathyroidism in contrast with those found in idiopathic hyperplasia of these glands.

The syndrome in the case presented appears to belong to the group classified as renal hyperparathyroidism and warrants consideration because of the age of the patient and the observations at autopsy.

REPORT OF A CASE

A 55 year old American housewife was first admitted Aug. 3, 1938, complaining of weakness, ease of fatigue, dyspnea on exertion and ulcerations of the legs.

She first noticed weakness in June 1937 together with ease of fatigue and dyspnea on moderate exertion. She noticed also some pain in the heels and legs while walking. Her activity gradually diminished from this time because of the progression of symptoms until June 1938, at which time her weakness was so marked that she was forced to go to bed. At this time she was treated for anemia and high blood pressure.

Ulcerations of the legs had commenced in May 1938 with a small shallow lesion on the right leg, which had increased in size until it involved an extensive area of

From the Department of Medicine, Temple University Medical School and Hospital.

1. (a) Albright, F.; Drake, T. G., and Sulkowitch, H. W.: Bull. Johns Hopkins Hosp. **60**:377, 1937. (b) Shelling, D. H., and Remsen, D.: *ibid.* **57**:158, 1935. (c) Karelitz, S., and Kolomozeff, H.: Am. J. Dis. Child. **44**:542, 1932. (d) Bass, M. H., and Pakter, J.: J. Mt. Sinai Hosp. **4**:882, 1938.

2. Castleman, B., and Mallory, T. B.: Am. J. Path. **13**:553, 1937.

the lateral surface. In June 1938 small discrete ulcerations commenced on the left leg and gradually increased in size until her admission into the hospital.

Of interest in her past history was scarlet fever at the age of 9. Since that time, whenever her urine was examined, as it was at approximately yearly intervals, she was told that it contained albumin. Nocturia (one to two times nightly) had been present from the age of 14, which she associated with a fall from a tree in which her side was injured.

She was married at 39 years. No pregnancies ensued. She was told that her pelvic organs were immature. Her best weight was 138 pounds (62.5 Kg.), in May 1938.

Physical Examination.—The patient was an alert, emaciated woman, with a moderately deformed chest and marked kyphosis of the dorsal part of the spinal column. She weighed 86 pounds (39 Kg.). The skin was pallid, dry, wrinkled and inelastic. The mucous membranes were somewhat blanched. There were shallow irregular granulating ulcerations of the skin on the lateral surface of the right leg, measuring 4 by 10 cm., and a few small shallow granulating areas of skin on the lateral surface of the left leg. She was edentulous. In the region of the cuspid area at the right in the lower jaw there was a hard nontender swelling, regular in outline, covered by mucosa and measuring 1 by 2 cm. The thyroid appeared slightly enlarged on the left, with some question as to an irregular lateral border. The eyegrounds showed arteriosclerotic changes, grade 2 of the hypertensive type. The thorax was deformed, presenting a typical pigeon breast appearance. The lungs were normal.

The heart appeared moderately enlarged to the left. The rhythm was regular; the rate, 88. There was a long rough high-pitched systolic murmur heard over the entire anterior wall of the chest, most marked over the apex and base of the heart. The murmur was transmitted into the vessels of the neck. A systolic thrill was present over the apex. The peripheral vessels, including the radial, dorsalis pedis and posterior tibial arteries, were of the calcified pipestem variety. The brachial, temporal and femoral arteries were markedly sclerotic and tortuous. The blood pressure was 186 systolic and 94 diastolic in both arms. The blood pressure and pulse rate were approximately unchanged during her stay.

The kidneys could not be palpated with certainty, perhaps because of the marked distortion of the chest and vertebrae. The liver and spleen were not palpably enlarged.

The urine showed only traces of albumin and no formed elements. The specific gravity remained fixed between 1.005 and 1.010 on concentration tests. The excretion of phenolsulfonphthalein, given intramuscularly, was less than 5 per cent in two hours. The urea clearance was reported as 12 per cent of normal.

There was marked hypochromic microcytic anemia, with 5.5 Gm. (34.7 per cent) of hemoglobin and a red blood cell count of 2,460,000. The leukocytes numbered 11,400, 64 per cent of which were polymorphonuclear cells. The bleeding time was one and one-half minutes; the coagulation time, five minutes. The blood platelets numbered 195,000.

The blood urea nitrogen was 68 mg. in 100 cc.; creatinine, 2.3 mg.; blood sugar, 71 mg. and cholesterol, 182 mg. The carbon dioxide-combining power was 30 volumes per cent. The serum calcium was 12.53 mg. and the serum inorganic phosphorus 5.28 mg. in 100 cc.; the phosphatase activity was 11.56 Bodansky units. The total serum protein was 4.66 grams in 100 cc.

Roentgen studies of the mandible revealed a cystic area with general demineralization of the cranial bones. This led to a complete skeletal study, which revealed

general decalcification. In addition there was marked calcification of the smaller and larger arteries (fig. 1), including the thoracic and abdominal portions of the aorta. The walls of the trachea and bronchi showed marked deposits of calcium. In the femurs periostitis with calcification of the subperiosteal tissue was noted. There was marked distortion of the thorax with marked kyphosis in the midthoracic



Fig. 1.—Roentgenogram (October 1938) showing marked demineralization of the right femur and general calcification of the medium-sized arteries of the soft tissues of the thigh.

region, as well as deformity of the pelvis and moderate varus deformity of the femoral necks. The cardiac valves were dancing calcified shadows as visualized by the fluoroscope, and calcified coronary arteries were in evidence on the roentgen film (fig. 2).

Roentgenograms of the renal areas revealed small renal shadows with calcification of the arteries in the parenchyma as well as some calcium deposits in the walls of the renal arteries. An intravenous urogram showed little excretion. At no time during the examination could the dye be definitely visualized in either kidney.



Fig. 2.—Roentgenogram of the heart taken post mortem. The calcified coronary arteries and the dense calcium deposits at the aortic ring are clearly seen.

An electrocardiogram showed the RST segment lowered 1 mm. in all standard leads. These findings were interpreted to be on the basis of myocardial changes secondary to calcification of the coronary arteries, together with anemia. The QT interval was normal.

In view of the findings of renal insufficiency, general demineralization of bone and fibrous cystic osteitis, a forty-eight hour study of the calcium balance and

a biopsy of the muscle of the thigh were carried out. The study of the calcium balance, employing a neutral ash diet (table 1) revealed a marked negative balance with increased output of calcium in the feces. The remainder of the table comparing calcium and phosphorus balances in patients with hyperparathyroidism and normal persons is taken from tables shown by Albright, Sulkowitch and Bloomberg.³

The muscle taken for biopsy showed a curious type of myositis of a degenerative and chronic nature, which did not seem to be based entirely on vascular deficiency. Larger arteries and arterioles revealed extensive sclerosis of the Mönckeberg type with calcification of the media. There appeared to be no changes in the smaller arterioles and capillaries.

TABLE 1.—*A Comparison of: (1) Metabolic Data on a Patient with Secondary Hyperplasia of the Parathyroids Obtained During Four Three Day Periods on a Neutral Ash, Low Calcium Diet; (2) Data on Patient E. M. C. Obtained During One Three Day Period and One Two Day Period; (3) Data from Metabolic Studies on Normal Controls and on a Patient with Primary Hyperparathyroidism*

	Calcium					Phosphorus			
	Urine, Ce.	Urine, Gm.	Feces, Gm.	Intake, Gm.	Balance, Gm.	Urine, Gm.	Feces, Gm.	Intake, Gm.	Balance, Gm.
Secondary hyperplasia ³									
First 3 day period.....	5,030	0.12	0.42	0.27	-0.27	1.10	0.69	1.53	-0.26
Second 3 day period.....	5,420	0.13	0.40	0.29	-0.24	1.12	0.67	1.68	-0.11
Third 3 day period.....	5,870	0.10	0.45	0.30	-0.25	1.06	0.72	1.77	-0.01
Fourth 3 day period.....	4,100	0.09	0.38	0.21	-0.21	0.82	0.72	1.14	-0.40
Average.....	5,105	0.11	0.41	0.27	-0.25	1.02	0.70	1.53	-0.20
E.M.C.									
3 day period.....	4,170	0.202	0.664	0.372	-0.404	0.738	0.765	0.771	-0.732
2 day period.....		0.106	0.708	0.20	-0.61
Normal controls (J. Clin. Investigation 10:221, 1931)		0.13	0.32	0.33	-0.12	1.21	0.60	2.07	+0.26
Primary hyperparathyroidism (J. Clin. Investigation 8:229, 1930).....		1.31	0.19	0.31	-1.20	2.22	0.24	2.10	-0.36

Course.—During her stay in the hospital the patient was cheerful and alert. She suffered no distress except on two occasions when she had attacks of tachycardia persisting from one-half to one and one-half hours. Electrocardiograms showed paroxysmal auricular tachycardia, which was easily controlled by quinidine sulfate, 3 grains (0.2 Gm.) daily, on one occasion and 6 grains (0.4 Gm.) daily on another. The granulating ulcerations on her legs were entirely epithelialized at discharge, Nov. 26, 1938. They were believed to be of factitious origin. The findings on chemical study of the blood throughout her stay are shown in table 2. The difference in serum levels of calcium and phosphorus in the first two determinations is not understood. She had been given a high calcium, low phosphorus diet, 3,600 USP units of vitamin D daily, fluids to 3,000 cc. daily and cod liver oil ointment, together with ultraviolet radiation for her skin lesions. She improved somewhat and was able to be up and about the ward at the time of her discharge in November. At the time of her discharge the serum calcium was 10.75 mg. and the serum phosphorus 5.2 mg. in 100 cc.

3. Albright, F.; Sulkowitch, H. W., and Bloomberg, E.: Arch. Int. Med. 62:199, 1938.

At home during December 1938 she managed to do a small amount of housework and was able to visit friends for the first time in seven months.

During the first week of January 1939 she experienced another attack of rapid heart action with considerable dyspnea, for which she remained in bed for several days. Following this, she was able to walk about the house until the last week in January, at which time another attack of tachycardia and dyspnea with cough, which persisted for six hours, sent her back to bed for three weeks.

She was readmitted on February 22, alert, cheerful and without any obvious change in her appearance or in the results of physical examination from what had been noted at the time of her discharge in November, except that the cyst on the right ramus of the mandible appeared slightly larger and the ulcerations of the skin were completely healed.

The findings on chemical examination of the blood were essentially unchanged with the exception of an elevation of the serum phosphorus (7.2 mg.; table 2) despite a high calcium diet supplemented by 3,600 USP units of vitamin D daily. During March she was able to sit up for long periods and was without complaint except for occasional pains over the lower costal margins, which on examination

TABLE 2.—Values Obtained in Repeated Chemical Studies of Blood (Milligrams per Hundred Cubic Centimeters)

	8/4/38	10/6/38	10/11/38	10/20/38	11/17/38	2/23/39	3/22/39
Serum calcium.....	12.53	10.75	10.7	10.6	10.7	10.5
Serum phosphorus (inorganic)....	5.28	2.8	5.72	5.2	7.2	6.58
Phosphatase.....	11.56	9.84	17.3	2.40
Urea nitrogen.....	68.0	50.0	96.0
Nonprotein nitrogen.....	85.0	80.0	123.0	90.0
Creatinine.....	2.6	2.3
Serum protein.....	4.66	5.79	5.94	5.03
Cholesterol.....	182.0	269.0
Plasma carbon dioxide-combining power.....	30.0	33.0	32.0	26.0
Blood chlorides.....	544.0	582.0

appeared to be bone tenderness. The blood pressure ranged between 168 systolic and 80 diastolic and 206 systolic and 94 diastolic on many readings. The pulse rate ranged from 86 to 94 per minute. During March, because of her relative well-being with chronic acidosis, as well as the fact that the thyroid gland, especially on the left, appeared moderately enlarged, basal metabolic rates were determined on three occasions at weekly intervals. The readings were plus 34, plus 40 and plus 49 per cent. At this time there were no signs of cardiac failure.

During the first week in April auricular tachycardia developed with pulmonary edema on two occasions, both of which subsided within one-half hour on sedation and administration of quinidine sulfate. April 11 she died suddenly, with left ventricular heart failure.

During her second admission a seventy-two hour study of calcium and phosphorus balance revealed a marked negative balance of each element (table 1).

Autopsy.—The thyroid was found to be definitely enlarged, weighing 50 Gm. The capsule was smooth; the gland tissue appeared homogeneous. There was no evidence of cystic degeneration. On the posteromedial border of one lobe and on the posterior lateral border of the other lobe near the upper pole there was a brownish red nodular mass which showed a definite line of cleavage between itself and the gland. When these masses were removed there remained small craters in the thyroid tissue, covered by capsule. The nodules resembled enlarged parathyroid glands and measured 1.5 by 1.3 by 1.0 cm., and 1.7 by 2.0 by 0.9 cm.,

respectively (fig. 3). A search was made along the trachea and esophagus for other tissue resembling parathyroid structures, but none was found.

The pituitary showed definite enlargement; the anterior lobe was herniated through the diaphragm to present a surface about 1 to 1.5 cm. in diameter, which impinged on the optic chiasm. The entire gland appeared to be enlarged, but the preponderance was in the anterior lobe (fig. 3).



Fig. 3.—At the top is the enlarged pituitary. The thyroid, in the center, shows depressions in each superior posterior aspect, from which two nodules, suspected of being parathyroid glands, shown laterally on each side, were removed. Below is one of the small contracted kidneys, with a granular surface and evidence of two small retention cysts. The relative size of the organs shown is striking.

The ovaries were sclerotic and the uterus infantile. The pancreas and adrenals showed nothing of significance. The pineal gland was greatly enlarged, measuring 3 by 2 by 1.5 cm. The enlargement was uniform and appeared not to have

caused any mechanical interference. It was firm in texture and normal in color. No areas of calcification or cyst formation could be palpated.

There was apparent considerable decalcification of bone. The skull bones offered little resistance to the saw and had a rough, spongy, porous appearance. Their texture was such that they could be cut without difficulty with a knife. The ribs were very thin, resembling rather thin cardboard, and could easily be broken between the fingers.

The aorta and peripheral vessels showed an inordinate amount of calcification. The distal part of the thoracic portion of the aorta was a solid calcium shell which had to be cracked open by force, the intima and what appeared to be most of the media having been replaced by calcium deposits. Other large vessels in the mesentery stood out as rigid pipestem arteries.

The pericardium appeared normal. There was no exudate. The heart was moderately enlarged. It weighed 485 Gm. The left ventricle showed well defined concentric hypertrophy. The tone and color appeared good, and the wall, as well as the interventricular septum, measured 2 cm. in thickness. There was no evidence of myocardial infarction.

The mitral valve measured 9 cm. in circumference, and the leaflets showed a moderate amount of sclerosis and slight calcification. There were definite thickening and calcification of the ring. The aortic valve revealed an enormous amount of calcification, the ring being thicker than an ordinary lead pencil and completely calcified. The aortic leaflets were calcified into rigidity; the orifice admitted the entrance of a small finger, the circumference measuring 7 cm.

The circumference of the tricuspid and of the pulmonary valve was 13 and 8 cm., respectively. The appearance of these valves was entirely normal.

The coronary vessels showed marked calcification (fig. 2). Throughout their tortuous course they stood out above the epicardium and were rigid to palpation. The lumens of the vessels were considerably diminished, but at no point could complete occlusion be demonstrated.

The kidneys were definitely smaller than normal. The left measured 9 by 4.5 by 2.5 cm. and weighed 60 Gm. The right measured 9 by 5 by 2.5 cm. and weighed 65 Gm. They resembled kidneys in the terminal stage of chronic glomerulonephritis. The capsules were greatly thickened and stripped with difficulty, exposing a rough, granular firm pale surface. After each was sectioned, it was impossible to differentiate the cortical from the medullary portion. Most of the normal architecture of the pyramids was obliterated. There were several small cysts in each kidney, measuring 2 to 4 mm., filled with chocolate-like material.

The lungs, liver, gastrointestinal tract and spleen were not remarkable. The latter showed a few calcified arteries.

Microscopic Observations.—Heart: The myocardial fibers showed advanced degenerative change. Some were entirely replaced by fibrous connective tissue. The fibers which remained presented advanced nuclear changes and loss of striation and were separated by edema. Occasionally there were aggregations of lymphocytes, probably the response to degenerating muscle tissue. There were several small areas of calcification, which appeared to bear no relation to vessels.

Lungs: Other than marked edema, the histologic features were not abnormal.

Liver: Congestion and granular degeneration were noted.

Spleen: There was excessive fibrosis, typical of passive congestion.

Kidneys: The microscopic picture in the kidneys was that of severe third stage glomerulonephritis (fig. 4). No normal glomeruli could be found. Those which were probably still functioning at death showed great thickening of the capsule and proliferation of the fibrous and endothelial elements of the tuft, which caused narrowing of the vascular bed as seen with azocarmine stain. In these cellular

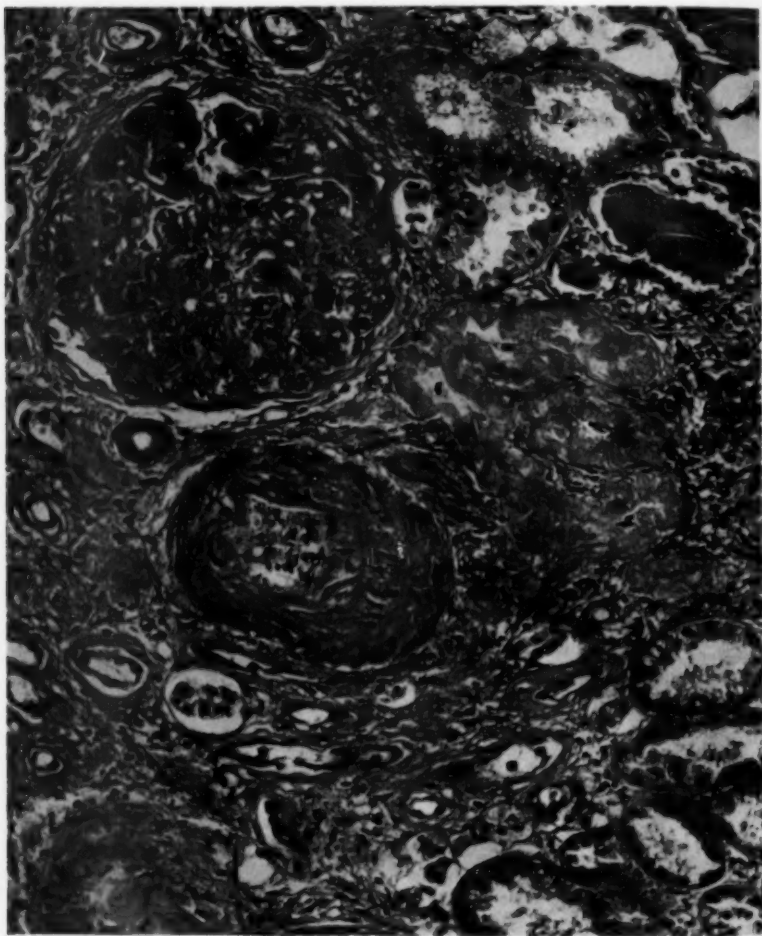


Fig. 4.—High magnification of renal tissue. Note sclerosis of medium-sized arteries and dilatation of the tubules. One glomerulus is entirely replaced by fibrous connective tissue, and another shows areas of fibrous necrosis.

tufts there were numerous areas of hyaline necrosis. Glomeruli presenting all degrees of scarring were found, the majority of them having been converted into solid fibrous masses. Many of the tubules had been completely effaced; the remaining ones showed great variation in size with irregular dilatation and plugging of the lumens with casts. Some of the tubules showed small areas of calcification.

No normal vessels were found. The medium-sized vessels showed pronounced medial hyperplasia with narrowing of their lumens. The small vessels presented hyaline necrosis of their walls with hyperplasia of the endothelial linings, which plugged the lumens. Many of the vessels showed areas of calcification in their walls. The interstitial tissue was dense and fibrotic. This was especially true

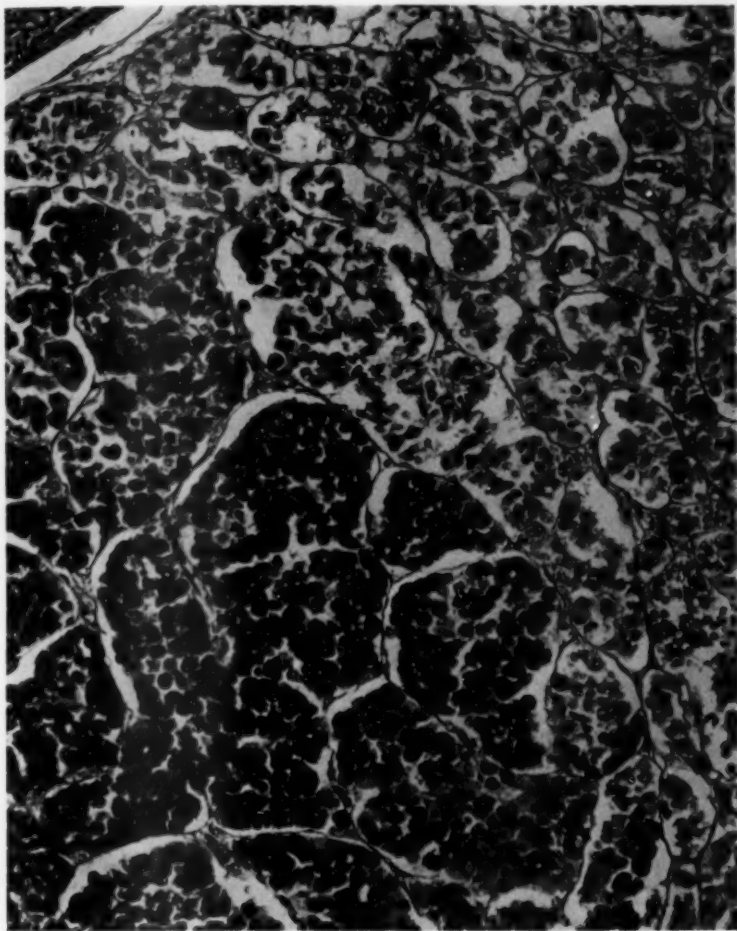


Fig. 5.—Photomicrograph of anterior lobe of the pituitary. The darker cells are basophilic by Rasmussen stain. There is a distinct contrast between the normal pituitary parenchyma and the basophilic adenoma.

toward the apices of the pyramids. There were areas which were densely infiltrated with lymphocytes.

Pituitary: The pituitary was considerably enlarged. This increase was apparently due to basophilic hyperplasia of the anterior lobe. In one region these cells were confined to a nodule measuring approximately 2 mm. in diameter,

forming a well demarcated basophil adenoma (fig. 5). The vessels of the anterior lobe were congested with blood. The posterior lobe was normal in appearance.

Bone: The ribs showed remarkable thinness. The calcium loss was diffuse.

Pineal Gland: The gland was definitely enlarged for the age of the subject. This increase was apparently due to hyperplasia of the parenchymal cells (fig. 6).

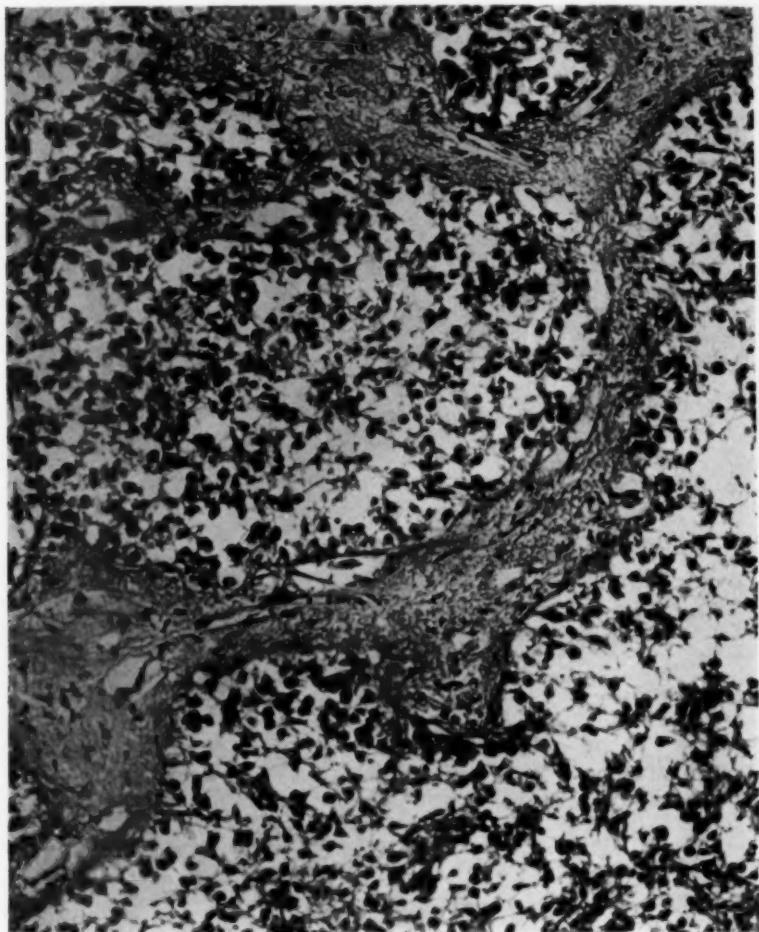


Fig. 6.—Photomicrograph of the pineal gland. Note the hyperplasia of the parenchymal tissue.

This hyperplasia was at the expense of the interlobular tissue. There was less calcification than one would expect to find in the normal pineal gland at this age.

Nodules on Posterior Surface of Thyroid: The nodules showed moderately hyperplastic thyroid tissue in multiple sections. There was an abundance of colloid tissue, which took the stain normally. The lining cells of the acini were cuboid but showed no evidence of changes found in thyrotoxic states (fig. 7).

Thyroid: The histologic appearance of the thyroid was similar to that of the nodules.

Adrenals, Pancreas: The adrenal and the pancreas showed nothing of abnormal character.

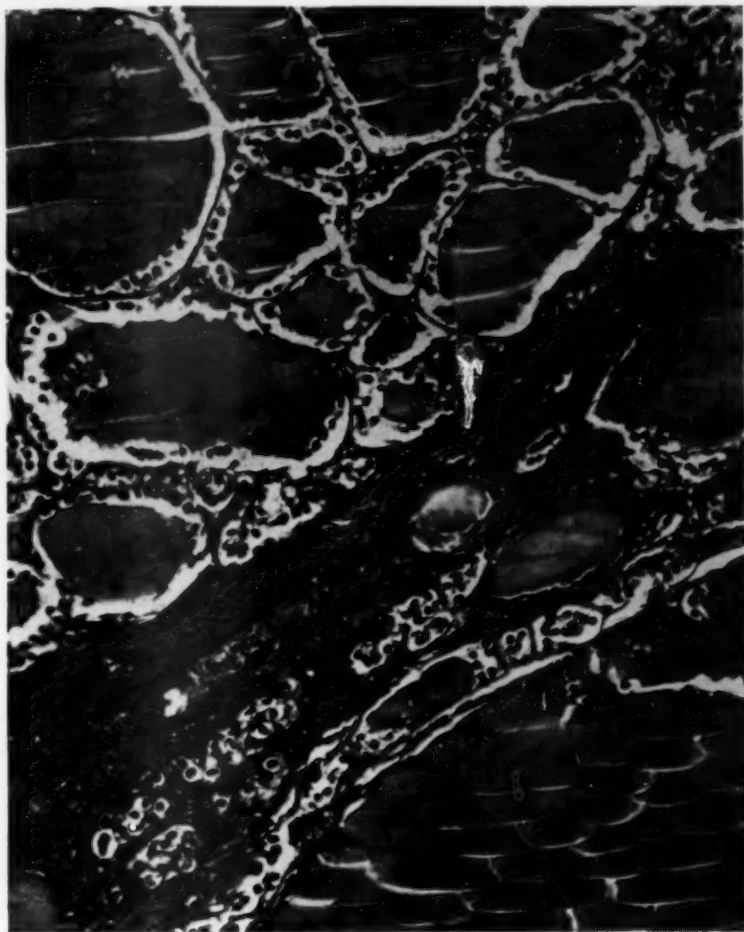


Fig. 7.—Photomicrograph of one of the nodules from the posterior surface of the thyroid. On gross examination these nodules were thought to represent parathyroid gland. Microscopically, the structure of thyroid is evident.

COMMENT

This case meets the clinical and metabolic criteria for the diagnosis of renal hyperparathyroidism. The long-standing albuminuria, the decompensated renal function, the metastatic calcification and the general skeletal demineralization gave rise to the clinical impression. Verifica-

tion by the normal value for serum calcium despite a low value for serum protein and a high value for serum inorganic phosphorus, and a negative calcium balance appear to establish the diagnosis. The slight increase in phosphatase activity also coincides with findings in renal hyperparathyroidism.

At autopsy nodules were found at the posterior superior poles of the thyroid gland, corresponding in location to those found by Shelling and Remsen^{1b} and by Albright, Drake and Sulkowitch,^{1a} as shown by the illustrations accompanying their findings in cases of renal hyperparathyroidism. The size of these nodules also approximates that reported in parathyroid hyperplasia secondary to long-standing renal insufficiency. There was a distinct line of cleavage between them and the thyroid gland. Histologically, however, the nodules were composed entirely of thyroid tissue. A careful search was made along the trachea and esophagus and in the mediastinum for other tissue resembling this. Neither this type of structure nor any that appeared to be normal parathyroid was found.

It is entirely conceivable that normal-sized parathyroid glands were overlooked. Platt and Owen⁴ reported a case of renal dwarfism with calcification of arteries and marked erosion of bone in which there was no enlargement of the parathyroids.

The marked skeletal demineralization and the metastatic calcification found may have been due to both the long-standing, moderately severe acidosis and the increased thyroid activity. Although Aub and Bauer⁵ showed that thyroid extract causes increased excretion of calcium and phosphorus, and it is well known that demineralization of bone follows long-standing thyroid hyperactivity, the part played by the hyperplastic thyroid in this instance is difficult to evaluate in the presence of chronic renal insufficiency and the accompanying chronic acidosis. The enlarged thyroid with the increased basal metabolic rate were not especially associated with clinical findings of thyroid hyperactivity, although the patient did appear to be at all times more alert than others noted in chronic acidosis accompanying slow renal failure. Pollack and Segal⁶ noted no signs of thyroid hyperactivity in a case they reported in which there were found chronic renal disease and hyperplasia of the thyroid and parathyroids with a greatly increased basal metabolic rate.

The extraordinary observations in the postmortem study were the great enlargement of the hyperplastic pineal gland and the enlargement of the pituitary—the latter as a result of hyperplasia of the anterior lobe, in which was found a small basophilic adenoma. In none of the reports on renal hyperparathyroidism is mention made of any abnormal

4. Platt, R., and Owen, T. K.: *Lancet* **2**:135, 1934.

5. Aub, J. C., and Bauer, W.: *J. Clin. Investigation* **7**:97, 1929.

6. Pollack, H., and Segal, S.: *J. Mt. Sinai Hosp.* **2**:270, 1936.

finding relative to the pineal gland, and in only a few is it stated that the pituitary was involved.⁷

Molineus⁸ suggested the possibility of some relationship between a large basophilic adenoma of the pituitary and diffuse adenomatous hyperplasia of the parathyroids in a case of osteitis fibrosa cystica, and Erdheim⁹ noted basophilic adenoma at autopsy in 2 cases of osteitis fibrosa cystica. Cushing¹⁰ found two slightly enlarged parathyroid glands in a case in which calcium and phosphorus excretion were normal but in which a small basophilic adenoma was present. In this instance the parathyroids were considered by Hertz and Kranes¹¹ as hyperplastic, according to the criteria of Erdheim. Pappenheimer¹² and Hertz and Kranes¹¹ showed that injection of anterior pituitary extract will cause enlargement of the parathyroid glands in rabbits. Chown and Lee¹³ in light of their observations are of the opinion that some conditions which are designated as renal rickets may have a pituitary-diencephalic malfunction as their etiologic basis. The relation between the enlargement of the pineal gland, the hyperplasia of the anterior lobe of the pituitary, containing the basophilic adenoma, the decalcification, the metastatic calcification and the chronic renal disease is not understood.

SUMMARY

A case of chronic glomerulonephritis with long-standing renal insufficiency, widespread metastatic calcification and general skeletal demineralization is presented which clinically appeared to be a case of renal hyperparathyroidism. Chemical study of the blood, tests of renal function and investigation of the calcium and the phosphorus balance verified the clinical impression.

Nodules of tissue which from their location, color and size were thought to be of parathyroid origin were found histologically to be moderately hyperplastic thyroid tissue. Parathyroid tissue was not found.

The unusual pathologic features were great enlargement of a hyperplastic pineal gland and hyperplasia of the anterior lobe of the pituitary due to basophilic adenoma.

7. Langmead, F. S., and Orr, J. W.: *Arch. Dis. Childhood* **8**:265, 1933. Shelling and Remsen.^{1b}

8. Molineus, L.: *Arch. f. klin. Chir.* **101**:333, 1913.

9. Erdheim, J.: *Rachitis und Epithelkörperchen*, Monograph aus der Kaiserlich-Königlichen Hof- und Staatsdruck, Vienna, 1914, p. 262.

10. Cushing, H.: *Arch. Int. Med.* **51**:487, 1933.

11. Hertz, S., and Kranes, A.: *Endocrinology* **18**:350, 1934.

12. Pappenheimer, A. M., cited by Park, E. H., and Eliot, M. M.: *Renal Hyperparathyroidism with Osteoporosis (Osteitis) Fibrosa Cystica*, in Brennemann, J.: *Practice of Pediatrics*, Hagerstown, Md., W. F. Prior Company, Inc., 1937, vol. 3, chap. 29, p. 7.

13. Chown, B., and Lee, M.: *Am. J. Dis. Child.* **53**:117, 1937.

EFFECTS OF AN ANTERIOR CALLOSAL GLIOBLASTOMA MULTIFORME ON THE ENTIRE BRAIN

MYRTELLE M. CANAVAN, M.D.

BOSTON

An English-born citizen of the United States was a clergyman in a small New England town. His principal diversion was gardening. When casually observed in May 1937, he seemed calm under the trying circumstance of having an only son admitted to a hospital for patients with mental disease. He gave at this time the impression of being in good health, and presented an excellent and cultivated appearance, but was dominated by his wife. He was quiet in manner.

In the early part of May 1938, when 64 years of age, he began to vomit bile in the mornings, and frequently his dinner was interrupted by similar attacks, but he had no nausea and he would continue the meal. He was troubled also with frequent micturition, which he believed was due to prostatic disease. His wife observed that he was no longer interested in his hobby of gardening, that he did everything slowly, such as taking an hour to shave, and was grateful when not hurried. He seemed to have lost the sense of time and often inquired what hour of day it was. When asked if he had a headache he would reply, "I have a slight headache," but would never remark about it spontaneously.

On May 29 he was fifteen minutes late for his Sunday service at the church, and on the next Sunday, June 5, not only was he late, but he stopped talking before his sermon was finished, and his wife signaled the organist to proceed with the music to cover the embarrassment of the incident.

On June 9 he left for the University Hospital in Iowa City to have a complete physical examination. During the journey he vomited on several occasions. He was admitted to the clinic on June 18.

Neurologic examination disclosed bilateral papilledema, equal grasp reflexes and tremor. He also had more active left-sided signs of tongue deviation, adiado-kokinesis and knee jerk. There was an excess of protein in the spinal fluid, the colloidal gold reduction was 1111222000, and there was an increased number of white cells, mainly polymorphonuclear, in the circulating blood. Two encephalograms were made. During the tests he became comatose and was placed on the dangerously ill list, but recovered. By this time he was confused, indifferent, bland, showed perseveration and became incontinent of bladder and bowel. A diagnosis of tumor of the frontal lobe was made.

He was referred, on July 7, to the University of Chicago, where he was examined by Dr. Percival Bailey. A ventriculogram disclosed occlusion and downward displacement of the anterior horns of both lateral ventricles. Dr. Bailey made a diagnosis of tumor of the corpus callosum, probably glioma and in any event inoperable.

His final care was undertaken at the Northampton State Hospital on July 15. At this time his pupils did not react to light or accommodation, the disks showed edema, and the left-sided signs continued. The mental picture was one of confusion; there was a fixed stare. The patient could sit up to eat but slept most of the time and remarked that he would be "dead the next time the doctor comes."

He comprehended with difficulty, spoke short sentences in a low voice, with an effort, was disoriented for place and was incontinent. He had no headache or vomiting, and no convulsions until six days before death, when they occurred for four days and nights. The last two days he was comatose, and he died

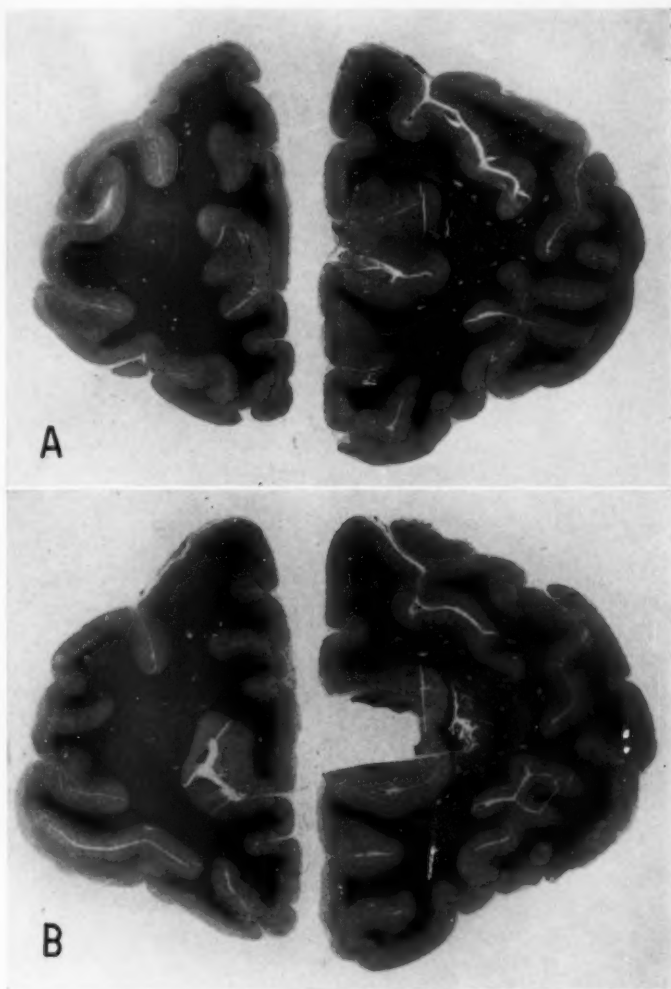


Fig. 1.—*A*, prefrontal section (natural size) stained by Weigert's method for myelin sheaths. The right hemisphere is larger and the white matter more vascular. *B*, section 0.025 cm. posterior to that shown in *A*. Note the inequality of the hemispheres and the increased vascularity of the white matter.

August 30, four months after his first symptom of vomiting. It may be noted that his brother had also died of a tumor of the brain.

The autopsy was made by the staff. The brain was fixed in solution of formaldehyde U. S. P. and after a few days was referred to me for study. It weighed

1,580 Gm. and measured 18.5 cm. in length, 15.75 cm. in width and 9 cm. in height. The basilar vessels were prominent but not sclerotic. Pressure effects were present in the orbital and piriform lobules and around the medulla. Particularly did the orbital surface bulge downward on the right more than on

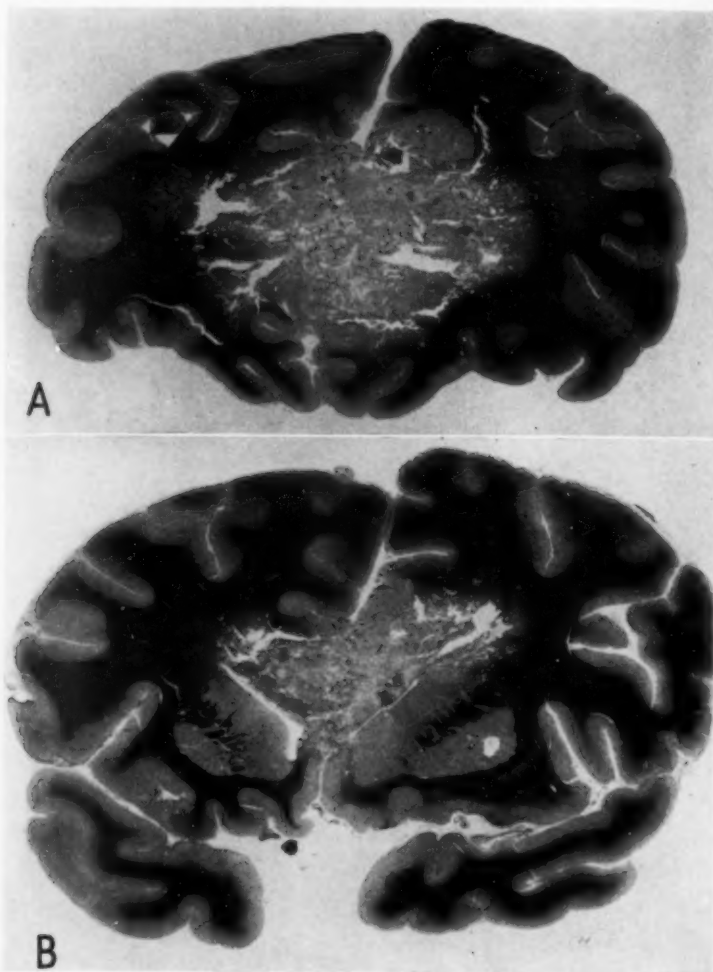


Fig. 2.—*A*, frontal lobes, showing a large central mass of tumor. Note the disappearance of the mesial cortex and the vascularity of the white matter at the periphery of the tumor, on both sides. *B*, section at the level of the anterior part of the temporal lobe. Note the larger hemisphere on the right. The central tumor is destroying the corpus callosum and the septum pellucidum. The margin of the tumor is necrotic, and the peripheral white matter is vascular.

the left. The corpus callosum as seen between the hemispheres presented a convex line for 5 cm. between the vertex and frontal poles.

Coronal sections were made. The first, 3 cm. from the prefrontal tip, disclosed on the right an area of spongy tissue, measuring 3.5 by 4 cm., which was

dull yellow with a pink border. In its center was a cavity with a ragged edge. This lesion involved the white matter and the mesial but not the lateral cortex. A similar smaller mass, without a cavity, occupied the left frontal region.

The second section passed through the middle of the olfactory tract. Here the growth was central, measured 4.5 by 3.5 cm. and caused compression of the lateral ventricles.

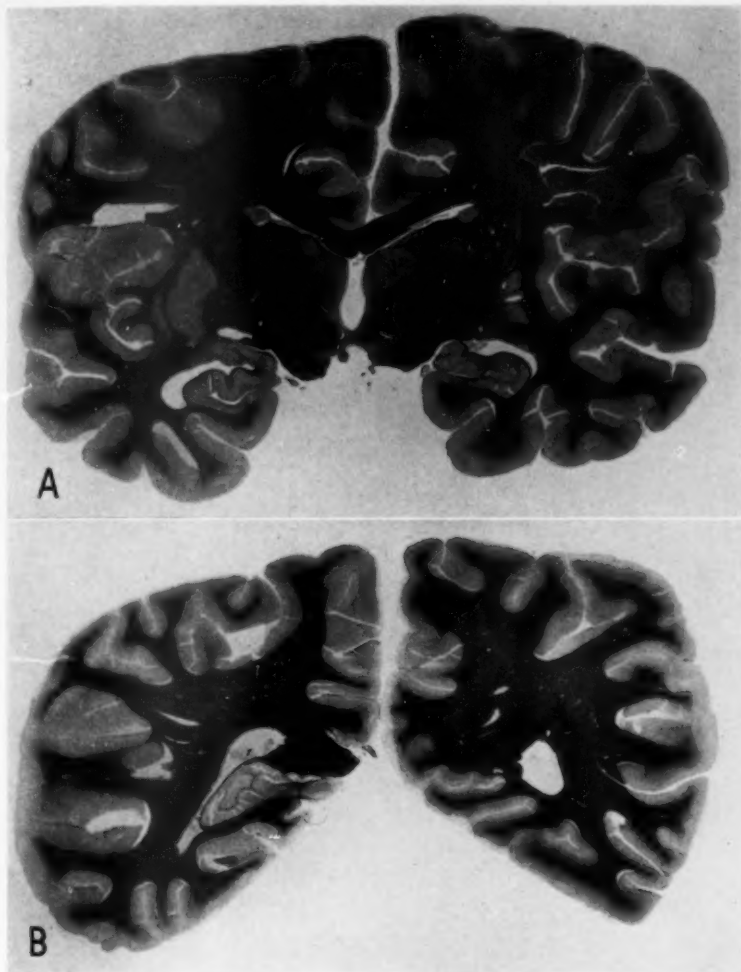


Fig. 3.—*A*, section at the level of the posterior portion of the hippocampal gyri. There is no tumor, and the vascularity is not increased. The left hemisphere is slightly larger in size. *B*, section through the parietal regions showing distorted hemispheres. The ventricles are unequal in size.

The third section was at the chiasm, where the mass invaded the corpus callosum and was limited to it and to the septum pellucidum and a mesial gyrus.

Further sections at 2 cm. intervals showed no tumor but showed some distortion and inequality of the hemispheres. The total extent of the grossly recognizable

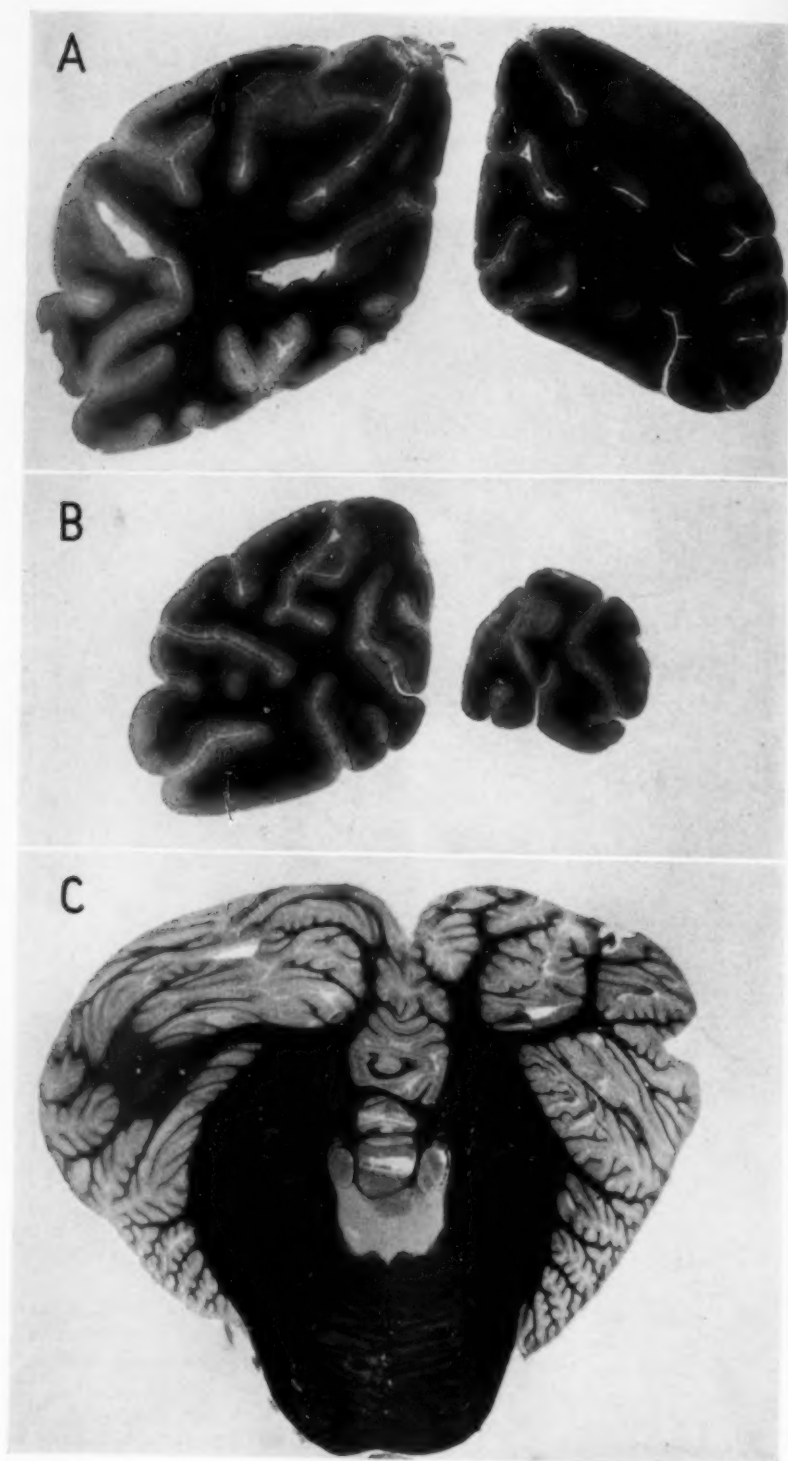


Fig. 4.—*A*, parieto-occipital sections. Note the larger left section. *B*, occipital sections. Note the greater size of the left section. *C*, section of cerebellum showing inequality of gray and white matter on the two sides and slight enlargement of the fourth ventricle.

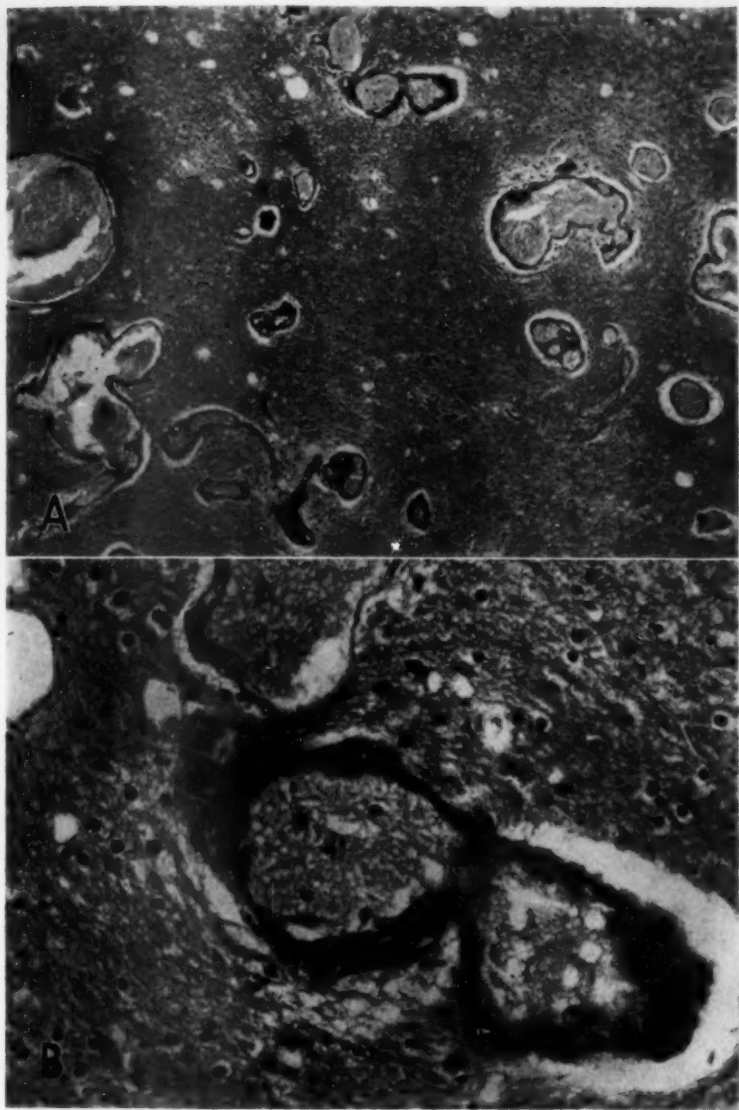


Fig. 5.—*A*, section from the right prefrontal region; hematoxylin and eosin stain; $\times 60$. Note the excessive vascularity as regards both arteries and veins. *B*, to show the endothelial lining; the twin vessels seen in the upper central region of *A*, hematoxylin and eosin stain; $\times 275$.

tumor was about 6 cm. anteroposteriorly, with a width of 2 to 4 cm., and the growth was located for the most part in the anterior portion of the corpus callosum and its adjacent white matter, chiefly on the right.

It seemed of importance to determine in what span in the brain the changes might be attributed to this rapidly growing tumor. For this reason total sections of the brain were cut from the seven blocks, at 50 microns, and were stained by the Weigert method for myelin sheaths (figs. 1 to 4 *B*, inclusive). As far as is known, a similar study has not been made before, and hence it is offered here as a garland.

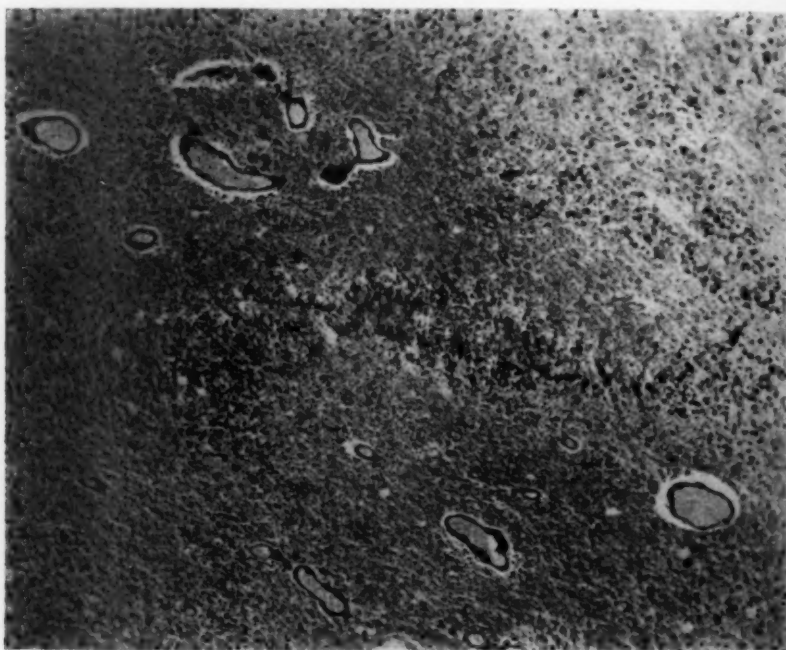


Fig. 6.—Pseudopalisading of nuclei at the periphery of a degenerated area in the tumor; hematoxylin and eosin stain; $\times 90$.

The edema of the brain, evident at the autopsy in the increased weight of 1,580 Gm. and represented by a pressure cone, may have been a terminal event, associated with the convulsions or the bronchopneumonia. It might, however, have been due to local pressure. The distortion of the brain, noted in figures 1 to 3 as right-sided and in figures 4 *A* and *B* as left-sided enlargement, might be considered as compensatory and of longer standing, but the left hemisphere of any brain is usually the larger throughout. The local replacement of brain by tumor tissue may be seen in figure 2. Attention is called to the vascularity

outstripping the tumor mass anteriorly (fig. 1). The forward growth of the vessels, preceding the tumor, not seen posteriorly, may have some relation to the branching of the anterior cerebral arteries, which supply the corpus callosum and most of the anterior lobes. The marked vascularity (fig. 5 *A*) is attributed to stimulation by the growth. The proliferation of the endothelial lining of the blood vessels in the neighborhood of the tumor is characteristic of glioblastoma multiforme (fig. 5 *B*). There is marked variation in the size and shape of the neoplastic cells. The tumor also shows typical pseudopalisading about the necrotic areas (fig. 6).

There is no appreciable evidence that demyelination was caused by the glioma except where the brain tissue was replaced by tumor.

SUMMARY

A man of 64 years was overcome by a rapidly growing tumor of the brain, which was recognized as of the corpus callosum and inoperable. The diagnosis was confirmed at autopsy. The tumor involved the anterior portion of the corpus callosum and adjacent white matter, particularly on the right side. Total sections were made from seven coronal slices of the cerebrum and one from the cerebellum. They were stained by the original Weigert method for myelin sheaths. The tumor mass replaced brain tissue focally, distorted the hemispheres and increased the vascularity forward of the tumor but did not destroy myelin peripheral to it.

Microscopic examination of the neoplasm revealed typical glioblastoma multiforme.

HEALED PULMONARY INFARCTS

BENJAMIN CASTLEMAN, M.D.

BOSTON

The subject of pulmonary embolism and infarction has recently assumed considerable prominence as the result of publications relating to the increased incidence, the reflex vasomotor origin and the use of heparin in the prevention of such occurrences. Despite the stimulation of interest in the subject, however, little has been written about what happens to an infarct, how it heals, and how to recognize it in its end stages, either grossly or histologically.

My co-workers and I have found that the most satisfactory method of preparing the lungs for postmortem examination is to inflate them after removal to their original size by instilling solution of formaldehyde U.S.P. through the trachea, clamping the trachea and immersing the specimen in the formaldehyde solution for a few days. The lungs are then sectioned in the presence of a roentgenologist, who has before him both antemortem and postmortem roentgenograms of the chest (the latter are taken at 7 feet [213.5 cm.] with the body suspended vertically against a cassette holder) and who insists on having every unusual shadow on the films accounted for anatomically. By this cooperative method we have been able to discover a larger number of lesions than would have been found in a routine examination of the lung.¹ One of the purposes of this work was to study the anatomic nature of linear shadows on roentgenograms, because most of them had been interpreted in the past, without pathologic confirmation, as atelectasis, interlobar pleuritis or empyema. We have shown, however, that many of these linear shadows were due to healing and healed infarcts which had shrunk asymmetrically and were projected as dense lines on the roentgen film. The recognition of these lesions as healed infarcts is therefore of considerable practical importance to the roentgenologist.

Since infarcts are always peripheral and involve the overlying pleural surface, localized chronic pleuritis may be the only gross evidence, before the lung is sectioned, of the presence of a healed infarct. There is no

From the Department of Pathology and Bacteriology, Massachusetts General Hospital.

1. Hampton, A. O., and Castleman, B.: Correlation of Postmortem Chest Teleroentgenograms with Autopsy Findings with Special Reference to Pulmonary Embolism and Infarction, *Am. J. Roentgenol.* **43**:305, 1940.

doubt that in a large number of cases healed pleurisy is assumed grossly to represent old tuberculosis or possibly pleurisy due to previous pneumonia and is not examined microscopically. We feel very strongly that in many of these cases the chronic pleuritis, if studied carefully, would show definite evidence of healed infarction. This does not apply, of course, to apical scars, most of which are of tuberculous origin.

The gross appearance of a healed infarct may often simulate a pulmonary fissure. It is therefore important to examine the base and margins of every anomalous fissure or sulcus to determine whether the abnormality may have resulted from an infarct which healed and produced a pleural dimpling or cleft. Occasionally the anomalous fissure is chiefly the result of atelectasis adjacent and in some obscure manner secondary to a small, shrunken, peripherally located healed infarct; i. e., the fissure is a localized horizontal focus of atelectasis extending from the depressed scar along the surface at the same level as the infarct. Many of these apparent anomalous septums are so inconspicuous when the lungs are examined at the autopsy table in their usual collapsed state that they would almost certainly be ignored. Distention of the lung with fixative makes the lesion prominent, because the lung surrounding the lesion becomes inflated and emphasizes the depressed cleft (fig. 1). The latter produces a linear band of increased density on the roentgenogram which is often helpful in calling attention to the lesion anatomically (fig. 2 *A*).

We have had several cases of healed infarction of the lung in which the clinical histories were inadequate for correlation with the anatomic observations. In a few, however, the clinical records gave confirmatory evidence that the healed lesions found at autopsy were infarcts. One of these cases was that of a 38 year old woman who was in the hospital four years before her death with signs and symptoms of congestive heart failure due to rheumatic heart disease with mitral stenosis. While in the hospital she had definite clinical symptoms and physical and roentgenologic signs of multiple pulmonary infarcts. During the next four years the patient was fairly well, greatly limiting her activity and being treated with digitalis, but following an infection of the upper respiratory tract cardiac failure developed, aggravated by acute rheumatic fever, and the patient died. At autopsy, scattered throughout the surfaces of both lungs were localized areas of chronic pleuritis, varying from 1 to 4 cm. in diameter. In the center of each of these was a round dimpled area, the depressions varying from 2 to 5 mm. in diameter. On section beneath these depressions there were apparent roughly perpendicular linear gray-white scars measuring from 0.5 to 2 cm. in depth and only 0.3 to 1 cm. in thickness (fig. 2 *B*). Microscopic examination of these lesions showed all the characteristics of the healed infarct to be described (fig. 3).

gradually shrinks and becomes smaller and denser. Ultimately, the organization having continued centripetally, there is fibrous replacement of all the infarcted tissue and the lesion may be considered healed. The smaller the infarct, the sooner it will organize completely. Before this final stage, i. e., during the healing stage, shadows of alveolar walls

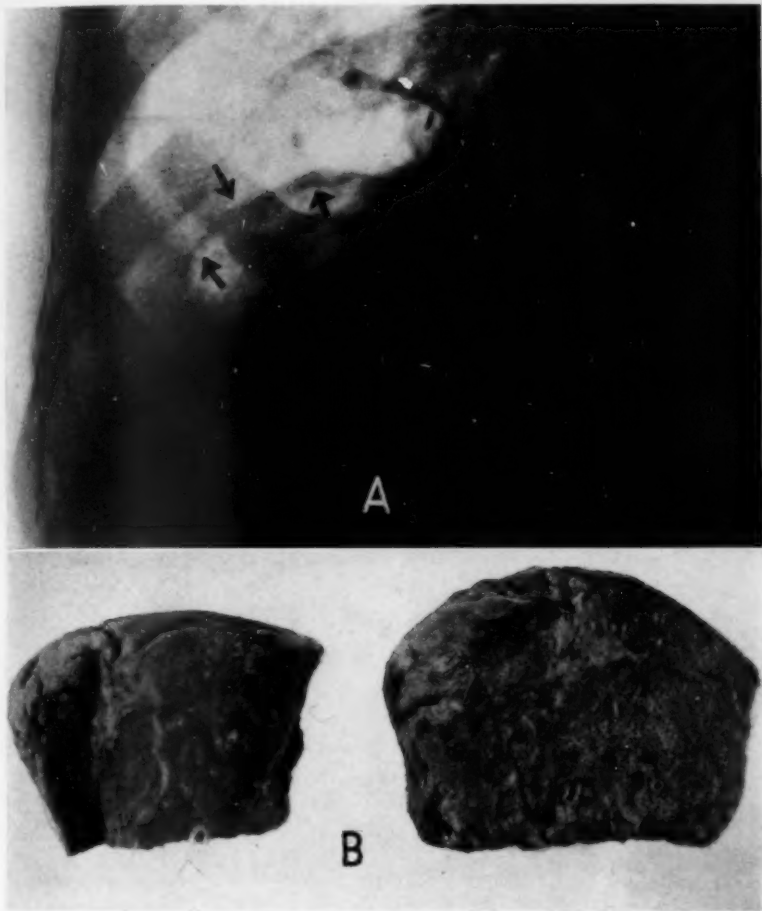


Fig. 2.—*A*, roentgenogram showing the linear shadow (note arrows) produced by the healed infarct demonstrated in figure 1. *B*, photograph of two healed infarcts. Note the characteristic pleural dimpling, the dilated bronchi within the lesion and the adjacent compensatory emphysema.

may still be identified, especially in elastic tissue-stained preparations, and even in the final healed stage the elastica is still present.

The microscopic appearance of a healed infarct may so closely simulate healed tuberculosis, organized pneumonia or any healed

infectious process that usually a diagnosis of healed infarction is not even considered. This may be readily appreciated since in all of these processes the fundamental lesion is fibrosis, and in hematoxylin-eosin preparations it would be almost impossible to distinguish one from the other. They are all composed of fibrous connective tissue with any degree of cellularity, some being much denser than others. Isolated

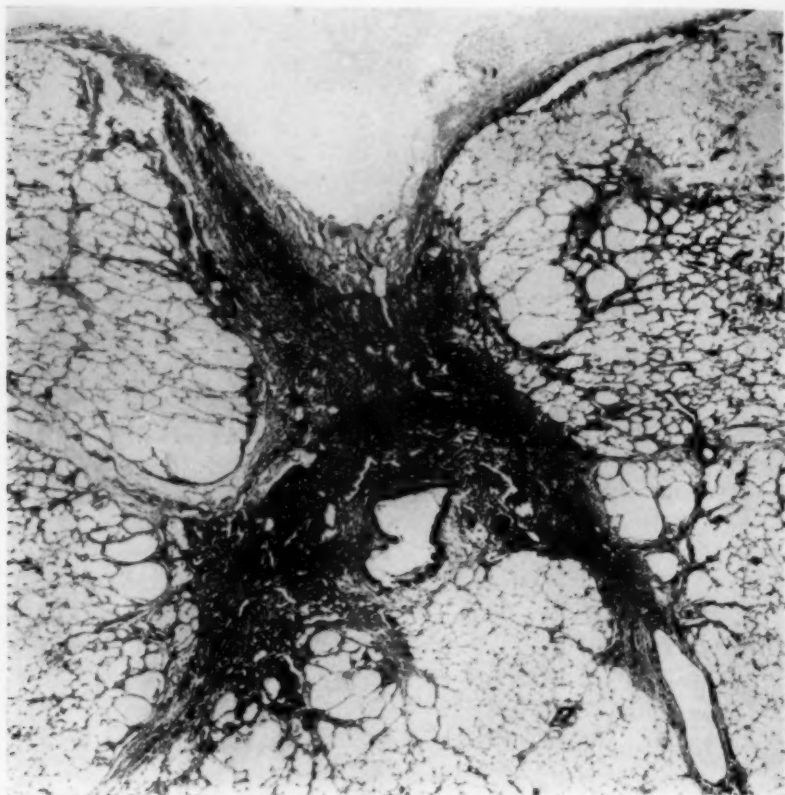


Fig. 3.—A low power photomicrograph of one of the lesions in figure 2B, demonstrating more clearly the pleural dimpling and neighboring emphysema. This is an elastic tissue preparation (elastica stains black) showing the preservation and condensation of the elastica.

bronchioles, usually moderately dilated, are often seen within the scarred areas. They have apparently remained viable, being supplied by the bronchial artery plexus which is normally present in the walls of the bronchi. Scattered deposits of hemosiderin may be observed in any of these lesions. Because of the decrease in volume in a fibrotic process, there is compensatory emphysema of the neighboring alveoli to allow

for spatial readjustment. Figures 4 *A*, 5 *A* and 6 *A* show photomicrographs of hematoxylin-eosin preparations of a healed infarct, organized pneumonia and healed tuberculosis respectively. It is only with elastic tissue stains (figs. 4 *B*, 5 *B* and 6 *B*) that any successful attempt at differentiation may be made. The arrangement and amount of elastica within the fibrous tissue are important criteria. In a healed infarct (fig. 4 *B*) the elastica is quite plentiful but no longer shows an alveolar pattern. Masses of elastic fibrils are crowded together in spiral, tangled, haphazard fashion and can best be described as simulating bunches of snarled curls of hair. Bland infarction therefore produces fragmentation and probably some proliferation of elastica but apparently never complete dissolution unless infection is superimposed—a very rare coincidence.

In ordinary types of organized pneumonia the alveolar walls remain intact and there is organization only of the alveolar exudate. An elastic tissue preparation (fig. 5 *B*) demonstrates very well the orderly alveolar pattern without destruction of elastica, and in this way the process is easily differentiated from healed infarction. In cases in which there has been destruction of alveolar walls, however, as in secondary abscess formation or pneumonia due to Friedländer's bacillus, most of the elastica is completely destroyed. The surrounding parenchyma here usually shows pneumonitis, which either resolves or organizes without destruction of alveolar walls, so that here also the alveolar pattern will remain in the elastic tissue-containing areas. There is no doubt that occasionally some healed infections will only partially affect elastic tissue and may therefore produce a lesion similar to a healed infarct. In these instances secondary thrombosis within the involved area is usually found to be the cause of the change and may be a differentiating feature.

Healed tuberculosis is so much more common than organized pneumonia and healed infarction that quite often any scarred lesion in the lung is empirically considered tuberculosis. Since infarcts are so rare in the apexes of the lungs, the characteristic dome-shaped healed tuberculous lesions² in these areas need not be confused with infarction. Although in other parts of the lung, especially at the margins of lobes or in the costophrenic angles, peripheral scars are much more apt to be healed infarcts, tuberculosis may not be excluded. Obviously, if caseation or tubercle formation is present in or near the scarred area, there is no doubt of the diagnosis. With reference to elastic tissue, caseation is analogous to abscess formation; i. e., no elastica will be found. In healed tuberculous lesions, however, as shown in a fairly large series examined, a definite alveolar pattern is retained in elastic

2. It is assumed here that apical scars are of tuberculous origin, although I am cognizant of the recent work of J. Davson and W. Susman (*J. Path. & Bact.* 45:597, 1937), who believe that they are the result of inhalation of siliceous dust.

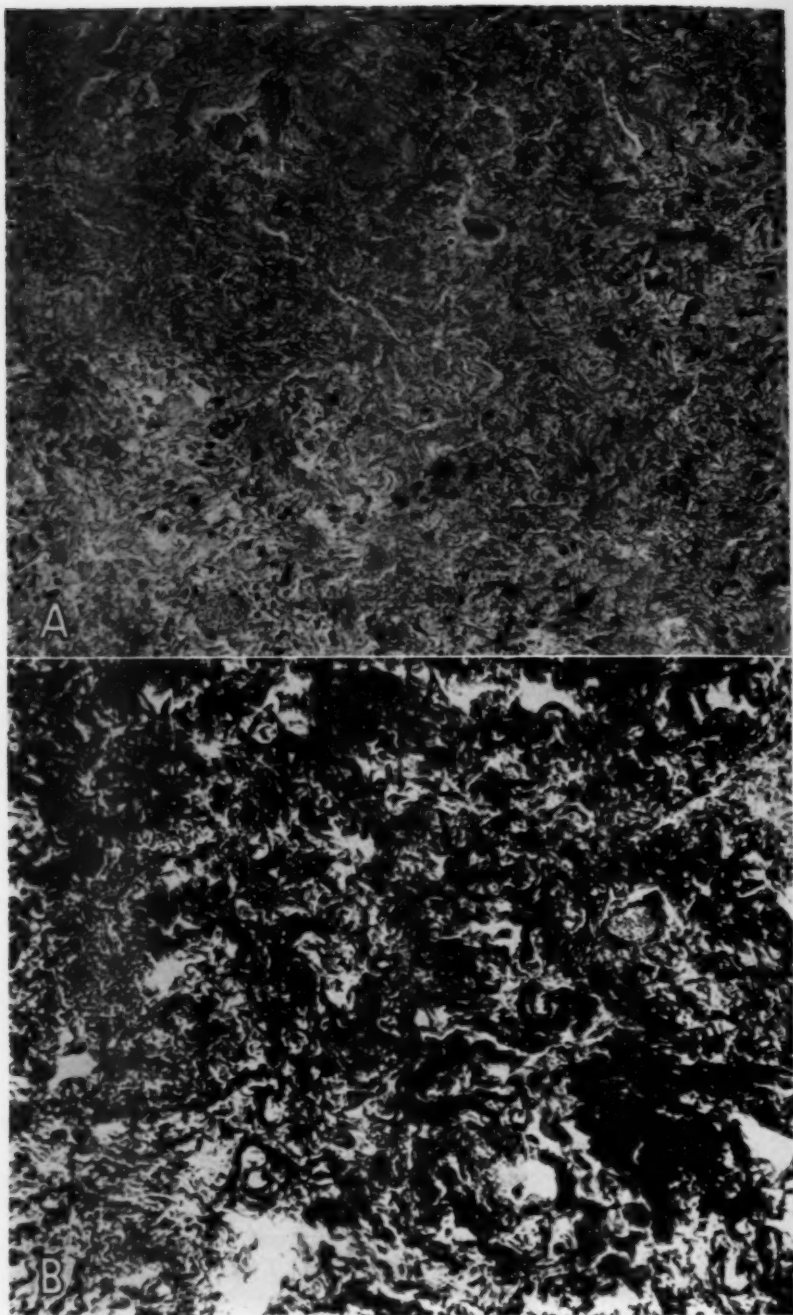


Fig. 4.—*A*, hematoxylin-eosin preparation of a healed infarct. Note the non-specificity of the lesion.

B, elastic tissue preparation of the same section, showing the haphazard arrangement of the elastic fibrils and the complete absence of an alveolar arrangement.

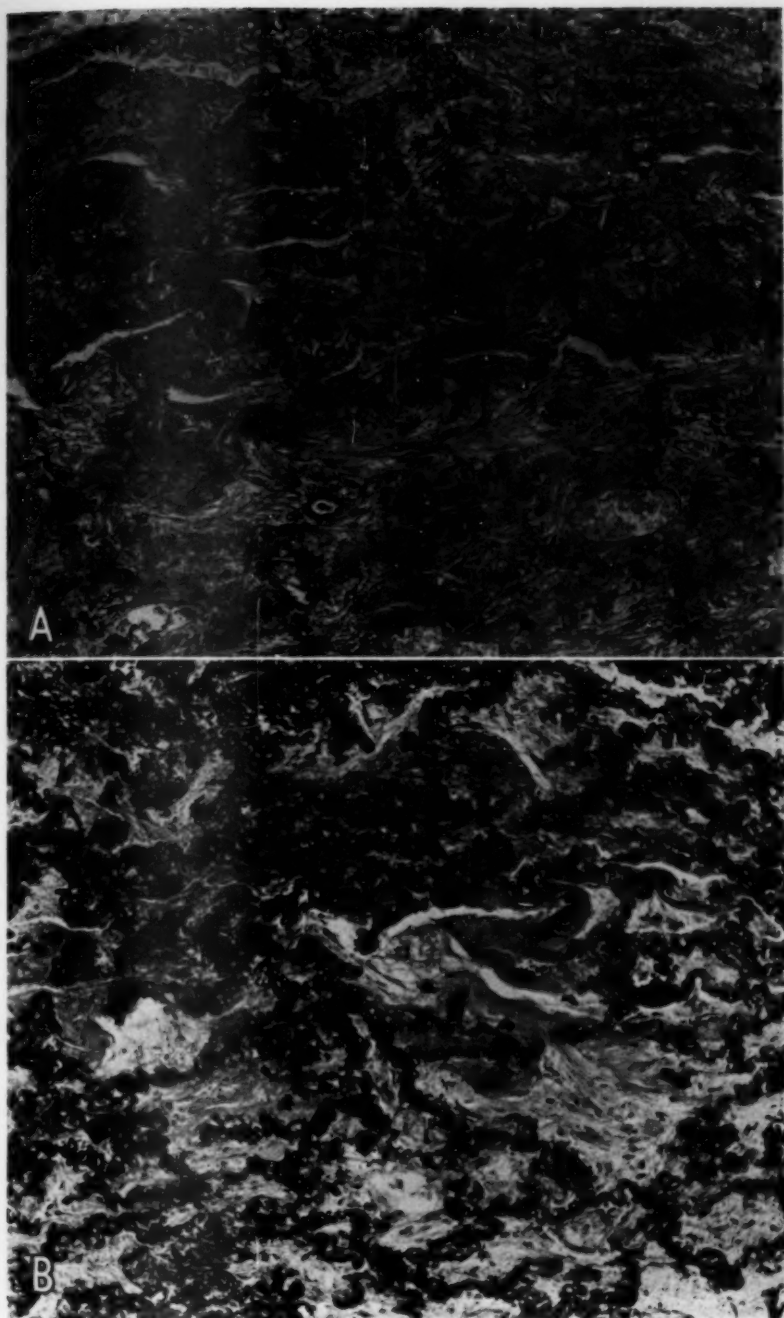


Fig. 5.—*A*, hematoxylin-eosin preparation of an area of organized pneumonia showing fibrosis. No alveolar walls can be observed.

B, elastic tissue preparation of the same section showing a definite alveolar pattern and fibrosis within the alveoli. Note also the proliferation of elastica.

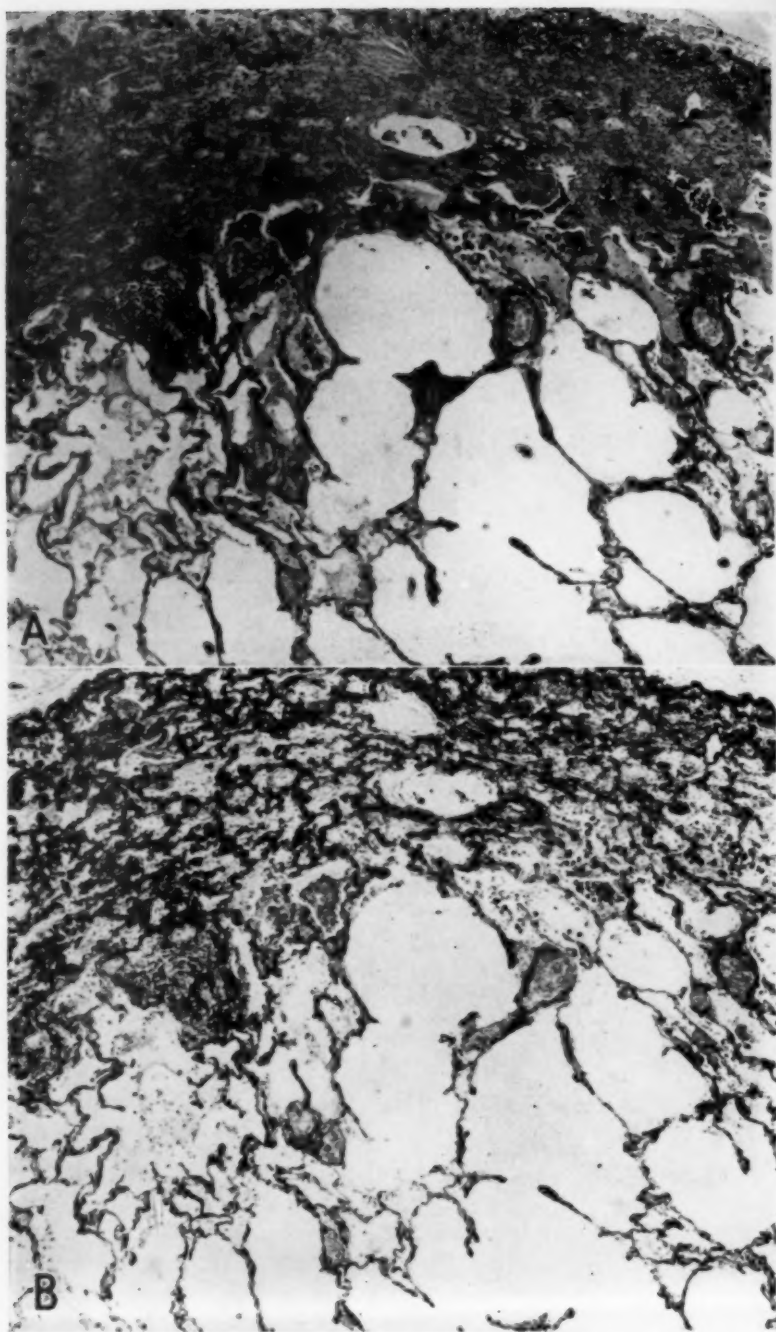


Fig. 6.—*A*, hematoxylin-eosin preparation of a tuberculous apical scar showing nonspecific fibrosis. Note subjacent compensatory emphysema.

B, elastic tissue preparation of the same section showing a well marked alveolar pattern.

tissue preparations, and there is organization tissue within the alveolar lumens (fig. 6). This is true not only in apical scars but also in tuberculous scars in other parts of the lung. These findings do not coincide



Fig. 7.—A low power photomicrograph of a recent infarct in a costophrenic angle, demonstrating the proximal location of the embolus and the characteristic hump shape of its cardiac margin.

with the statement in a recent paper by Davson and Susman²: “. . . it is generally admitted that in the presence of tuberculosis the elastic

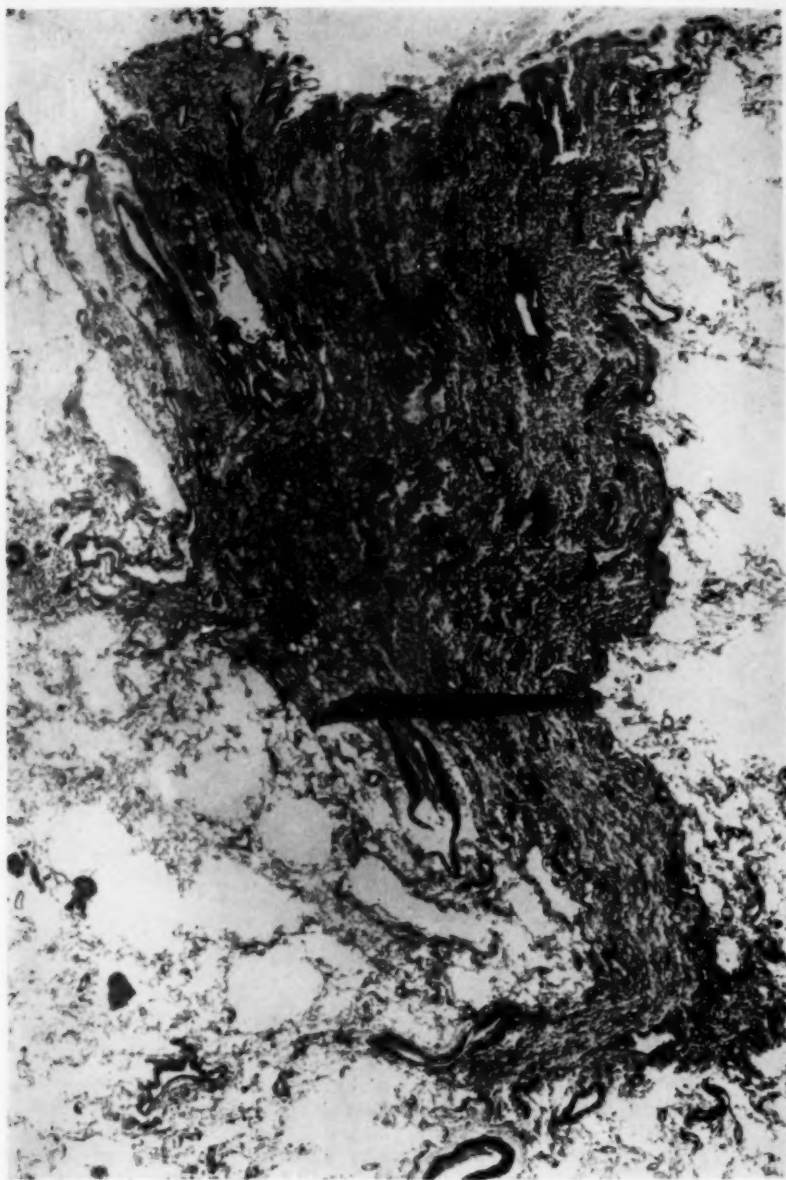


Fig. 8.—Low power photomicrograph of a cross section through a healed infarct. The organized embolus is located contiguous to the infarct. Note the marked condensation of the elastica.

framework of the lung is eventually destroyed and proliferation of elastic tissue is not a feature." Since apical scars do show an elastic framework, these investigators expressed the belief that such scars are not tuberculous in origin. I admit that when the tuberculosis is caseating and destructive the foregoing statement holds, but since the disease may be exudative within alveoli and simulate pneumonia,³ organization of this type of tuberculosis leaves the alveolar pattern

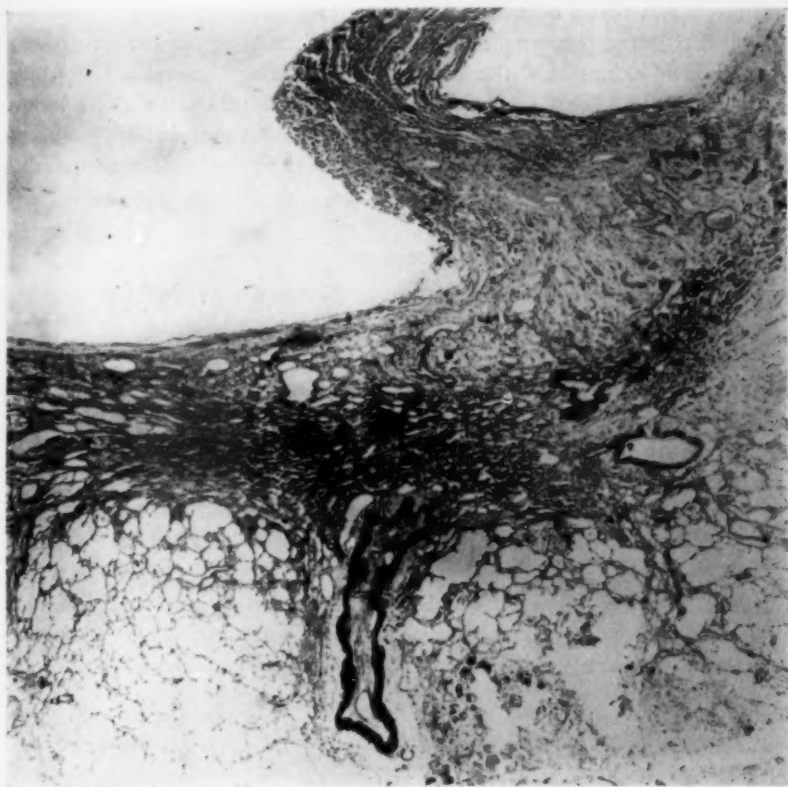


Fig. 9.—A low power photomicrograph of a section through the pleural tab in figure 1, showing the proximal site of the recanalized embolus.

intact and can therefore be differentiated from a healed infarct. Healed caseating or destructive tuberculosis does not show any appreciable amount of elastica and is therefore not confused with an infarct.

Another and very important criterion in the recognition of a healed infarct is the nature and location of the embolus. In a recent infarct

3. Jaffé, R. H.: *Arch. Path.* **18**:712, 1934. Ornstein, G. G.; Ulmar, D., and Dittler, E. L.: *Am. Rev. Tuberc.* **23**:223, 1931.

the embolus is usually found proximal to the infarct, although superimposed thrombosis may extend up to and within it (fig. 7). Apparently the lung in the region of the proximal portion of the embolus is supplied by neighboring branches of the pulmonary artery. The shape of the proximal or cardiac margin of the infarct is therefore convex toward the heart, producing a hump-shaped border. The subject of the shape of infarcts is fully discussed in a previous paper.¹

As the infarct heals the embolus also heals and may even canalize. If, therefore, a vessel containing an organized and very often recanalized thrombus is found proximal or contiguous to the apparent healed process, there is little doubt that the lesion is an infarct (figs. 8 and 9). If, on the other hand, an organized thrombus is found within the lesion, one should not immediately and unquestionably label the lesion an infarct, because it is well known that secondary thrombosis occurs within pneumonic and other infectious processes.

SUMMARY

Little attention has been paid to the recognition and incidence of healed pulmonary infarcts.

The method of choice in preparing the lungs for postmortem examination is to instill solution of formaldehyde U. S. P. through the trachea, inflating them to their original size, and to section them after fixation at this size.

Localized chronic pleuritis elsewhere than at the apexes, puckering and anomalous septums should always be suspected of being due to healed pulmonary infarcts.

The persistence and arrangement of the elastic tissue and the proximal location of the organized embolus are the two important criteria in differentiating the healed infarct from organized pneumonia or healed tuberculosis.

MYOEPIITHELIAL HAMARTOMA OF THE GASTROINTESTINAL TRACT

A REPORT OF EIGHT CASES WITH COMMENT CONCERNING
GENESIS AND NOMENCLATURE

B. EARL CLARKE, M.D.

Pathologist and Director of the Tumor Clinic of Rhode Island Hospital
PROVIDENCE, R. I.

Tumor-like masses composed of smooth muscle and epithelial components are occasionally encountered in the gastrointestinal tract. Certain histologic variations which occur in them have led to confusion in their classification in descriptive literature. This confusion is due to failure to appreciate their essential or basic pathogenesis. It is my purpose to record 8 cases which I believe illustrate their essential sameness and support the opinion that attempts to divide them are illogical and serve no useful purpose.

SUMMARY OF CASES

CASE 1.—A white youth of 18 years, a patient of Dr. Joseph Franklin, was first seen Feb. 5, 1933, with a chief complaint of epigastric pain, nausea, anorexia and constipation dating back only one month. Roentgen studies were done by Dr. Philip Batchelder, whose report reads: "In the region of the antrum of the stomach, just before the pylorus, there is a small rounded shadow of decreased density. This area is brought out on palpation of the stomach and does not show when palpation is stopped. The shadow has not been demonstrated in the many films taken following the fluoroscopic examination. The patient was examined twice by fluoroscope on successive days and showed the same findings at both examinations. The findings are those of a benign tumor of the antrum of the stomach, probably a polyp or a fibroma." At operation "on the anterior wall of the stomach 1½ inches (3.5 cm.) from the pylorus there was found a definite rounded tumor mass, about 1¼ inches (3 cm.) in diameter, apparently not involving the serosa and so far as could be determined, not involving the mucosa." An elliptic portion of the stomach, including the tumor, was removed. There was an uneventful postoperative recovery. The follow-up note from Dr. Franklin states: "This boy has had no further symptoms since operation. He has grown normally and is now married and in good health (November 1939)."

The specimen consisted of a portion of stomach wall measuring about 4 by 2 cm. The mucosa was intact. Beneath it was a rounded tumor mass measuring 2 by 2 by 1.5 cm. This when cut across appeared much like the normal muscle coat and blended with the normal muscle at the external aspect. It had a somewhat browner tint than the normal musculature. The rounded mucosal surface was sharply delimited, and the mucosa moved freely over it. No cystic spaces were observed grossly. Microscopic examination showed the mass to be composed of bundles of smooth muscle interlacing in a haphazard manner. The

line of demarcation between the tumor and the normal muscle was more definite than was apparent grossly, but there was not a true capsule. Scattered throughout the muscle tissue were numerous small ductlike spaces. Usually these were single, but occasionally small groups of them were held together by a scanty fibrous stroma, giving a glandlike appearance. The epithelial lining of these spaces was quite uniform, there being always a single layer of cuboid or low columnar cells. The nuclei were oval and basal, each occupying about one half of the cell volume. The cytoplasm was rather hyaline and stained deeply with eosin. The cell boundaries were quite indistinct. Sometimes a fibrous stroma was discernible about these ducts, while elsewhere the epithelial cells seemed to rest directly on the smooth muscle cells. There were a few small focal accumulations of lymphocytes, which seemed not to be associated with the ducts.

CASE 2.—A white boy of 15 years was admitted to the Rhode Island Hospital Aug. 27, 1937. The chief complaint was pain in the lower part of the abdomen and vomiting, of three days' duration. A diagnosis of acute appendicitis was made. Operation disclosed intussusception of the ileum with a gangrenous condition of the bowel. Resection and anastomosis were done. The patient died on the fourth post-operative day of acute peritonitis (confirmed at autopsy).

The surgical specimen consisted of a segment of ileum 58 cm. in length. The intussusception had been reduced. Seven cubic millimeters from one end of the specimen was a diverticulum 10 cm. in length and averaging about 2.5 cm. in diameter. At about its midpoint a polypoid mass with a broad base extended from the wall of the diverticulum into its lumen. This measured 2.5 by 2 by 1.5 cm. The entire diverticulum, including this mass and about 20 cm. of the adjacent ileum, was purplish red, soft and apparently infarcted. The histologic structure was obscured by the infarction. However, much pancreatic tissue could be recognized. This appeared everywhere to be well differentiated. No islets were demonstrable. The pancreatic tissue was divided into small lobules by wide bands of necrotic pink-staining tissue. Because of the necrosis, the presence of smooth muscle could not be definitely established.

CASE 3.—The patient was a white woman aged 52. She was a patient of Dr. Lucius C. Kingman, first seen by him Feb. 18, 1932, at which time she gave a history of having been treated "off and on" during the previous twelve years for "gall trouble." Her attacks consisted of pain in the epigastrium followed by vomiting, which relieved the pain. Lying down also gave relief. Physical examination disclosed nothing of importance. The Graham test was advised, but the patient refused and was not seen again until Feb. 17, 1938. During the six year interval she continued to have attacks as before described but with increasing severity. She was never jaundiced. At operation, a chronically diseased gall-bladder was removed, a peptic ulcer of the anterior duodenal wall 2 cm. from the pylorus was resected, and a small firm whitish mass in the anterior wall of the antrum was also resected. Convalescence was satisfactory, and there have been no further digestive disturbances.

The pathologic examination confirmed the diagnosis of chronic cholecystitis and duodenal ulcer. The specimen from the gastric wall measured 2.3 by 1 cm. On the mucosal surface was a peculiar umbilicated depression, 0.5 cm. in diameter and 0.5 cm. in depth. The mucosa turned down into it, and when cut across, was seen to be intact across the depth of the depression. Beneath this depression, apparently within the submucosa but fusing with the muscle wall below, was a flattened nodule, 8 mm. in diameter. Macroscopically, it appeared more like fibrous tissue than muscle. On microscopic examination there was found within the mucosa at the bottom of the depression a small lobule of pancreatic tissue. Below

this the nodule was found to consist of interlacing bundles of smooth muscle, scattered through which were other islands of pancreatic tissue, islands of Brunner's glands and undifferentiated ductlike spaces lined by cuboid epithelial cells. Included within two of the lobules of duodenal glands were small lymph follicles.

CASE 4.—Another patient of Dr. Lucius Kingman, a white woman aged 58, complained of severe pain directly after eating. There was no vomiting. Physical examination showed tenderness in the right upper quadrant of the abdomen. Roentgen studies of the gastrointestinal tract gave negative results except for

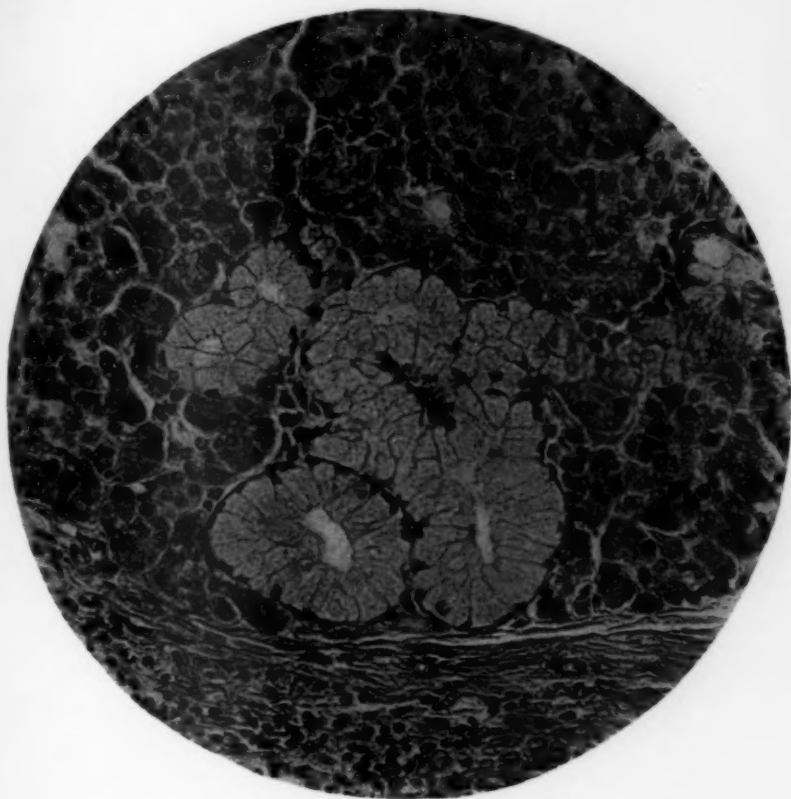


Fig. 1 (case 3).—A mixture of well differentiated Brunner's glands and pancreatic tissue. Below is smooth muscle infiltrated with lymphocytes.

"slow emptying of the gallbladder." At operation, July 22, 1938, a tumor "the size of a walnut" was found in the pylorus. The pylorus and part of the antrum were resected, and gastrojejunostomy was done. At last report, April 1, 1939, she was free of gastric symptoms.

The specimen consisted of a segment of stomach 6 cm. in length. Near the pyloric end was a rounded mass 1.5 cm. in diameter. This was situated between the mucosa and the serosa, both of which were intact, smooth and moving freely over the mass. On section the mass, although rather sharply delimited, was not encapsulated but merged with the muscular wall of the stomach. The cut surface

resembled that of the normal muscle but was more opaque. Microscopically, the mass consisted of tangled bundles of smooth muscle interspersed with numerous ductlike spaces. These varied in size and shape and were lined by a single layer of cells. These cells varied from an undifferentiated cuboid type with hyaline, eosinophilic cytoplasm to a tall columnar type with much clear cytoplasm, which was vacuolated. Cells of the latter type were sometimes in small groups, suggesting Brunner's glands. There was a small ulceration of the gastric mucosa directly over the mass, leaving a surface covered by granulation tissue. This was infiltrated



Fig. 2 (case 5).—A group of ducts showing progressive differentiation toward normal adult duodenal (Brunner's) glands.

with leukocytes of various kinds, including many polymorphonuclear neutrophils. Also, in the tumor mass, some of the ducts contained polymorphonuclear leukocytes, and some had lost their epithelial lining, forming small abscesses.

CASE 5.—A patient of Dr. Emery Porter, a white woman 39 years of age, was operated on June 15, 1939, for ectopic pregnancy. Before closing the abdominal wound, Dr. Porter reached up to bring down the omentum so that it would be under the incision. There was very little omentum, and in feeling for it he encountered a small mass at the pylorus. A small incision was then made over this region, and a small olive-shaped tumor was found just distal to the pylorus.

It was sharply outlined and firm but not hard. It moved freely beneath the serosa. A small portion was removed for pathologic study. The convalescence was uneventful. No history of any gastrointestinal disturbance could be obtained, and roentgen studies of the gastrointestinal tract were reported as yielding negative results.

The specimen consisted of two irregular-shaped pieces of pinkish gray translucent tissue, each measuring about 1 cm. over all. Two or three 1 mm. cavities were visible. Microscopic preparations consisted of smooth muscle, small

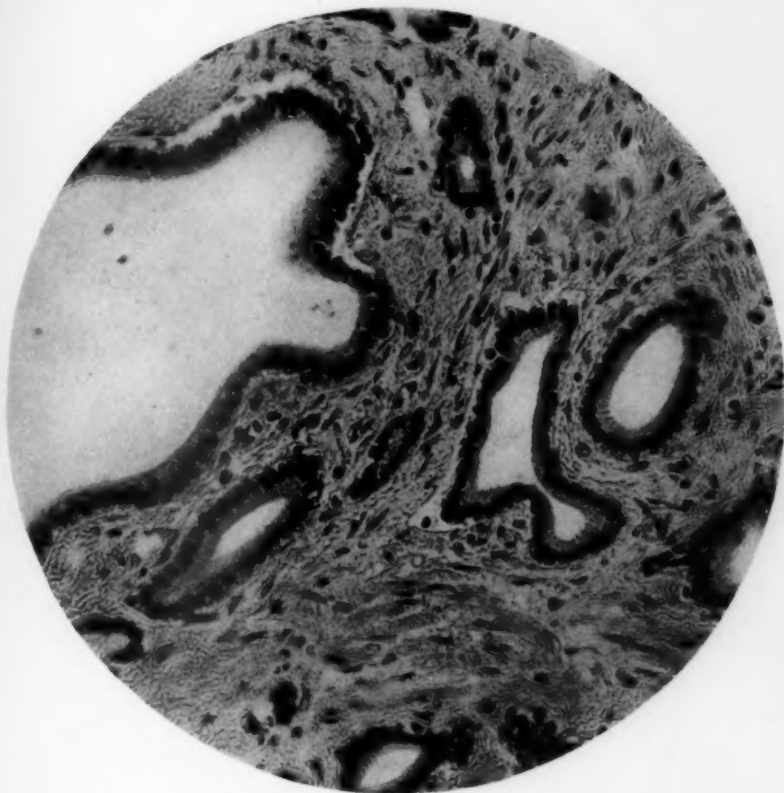


Fig. 3 (case 7).—Groups of cystic spaces lined by cuboidal or columnar epithelium and resembling pancreatic ducts or bile ducts. Between them is a stroma of fibrous and smooth muscle tissue.

ductlike spaces, lined sometimes by undifferentiated cuboid cells and sometimes by tall goblet cells, a few small groups of acini identical with Brunner's glands and one small island of definite pancreatic tissue.

CASE 6.—A white woman aged 31 was admitted to the Rhode Island Hospital Oct. 21, 1939. For one year she had suffered from discomfort, distention and belching after meals. Ten days before admission there was a sudden attack of severe epigastric pain which radiated to the right scapular region. This was accompanied by a chill and vomiting. Following this she became jaundiced.

Briefly, a diagnosis of gallstones was made, and cholecystectomy was done October 25. Pathologic examination confirmed the clinical diagnosis of chronic cholecystitis and cholelithiasis. In addition there was found in the wall of the gallbladder a rounded nodule 1.5 cm. in diameter. It was rather sharply delimited but not encapsulated. It appeared to bulge outward into the subserosal fat rather than into the lumen. Histologically, it consisted of interlacing bundles of smooth muscle, within which were numerous ductlike spaces lined with a single layer of rather tall cells with basal nuclei and considerable eosin-staining hyaline cytoplasm.

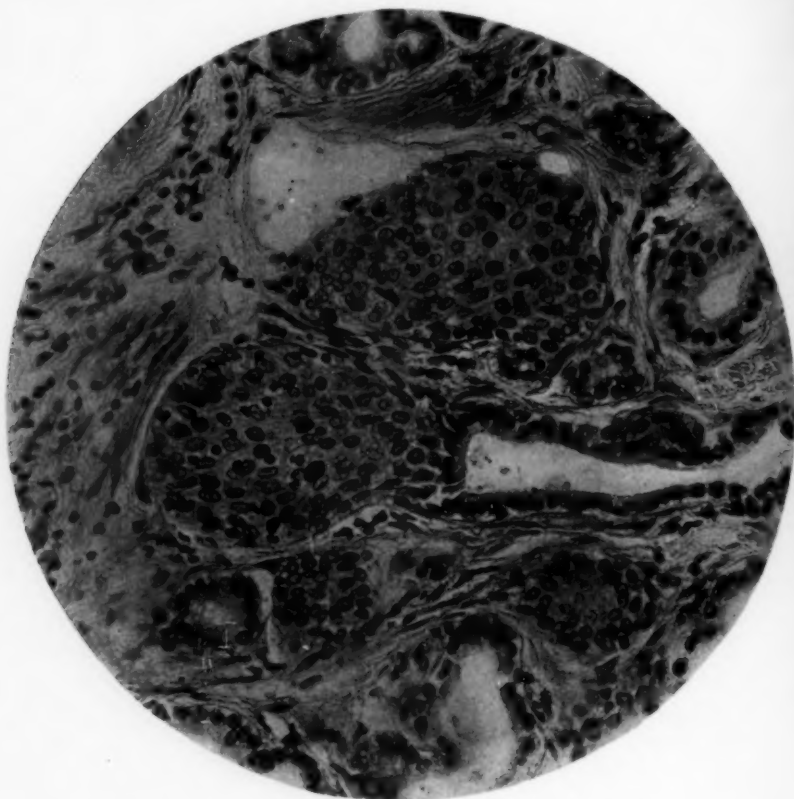


Fig. 4 (case 8).—Two solid epithelial buds are shown together with a few ductlike structures. Near the center the cells of the solid mass fuse with those of the duct lining.

These resembled the cells of the normal mucosa of the gallbladder but not more so than those in some of the other cases. There was no differentiation toward Brunner's glands or pancreas. The size and discreteness of the nodule appear to eliminate the probability of its being only an inflammatory inclusion of mucosal epithelium.

CASE 7.—A white man aged 64 was admitted to the hospital in coma and died shortly after arrival. At autopsy he was found to have hypertrophy of the prostate gland with urinary obstruction. As an incidental finding there was a

nodule 1 cm. in diameter in the jejunum. It lay within the muscle wall and bulged outward beneath the serosa. It was found to consist of numerous small ductlike spaces scattered between irregularly arranged bundles of smooth muscle cells. Most of these ducts were lined by a single layer of undifferentiated cuboid epithelial cells, but a few were lined by tall columnar cells, resembling the epithelium of pancreatic ducts or the mucosa of the gallbladder. The tumor was rather sharply delimited from the normal muscle. The overlying mucosa and submucosa were intact.

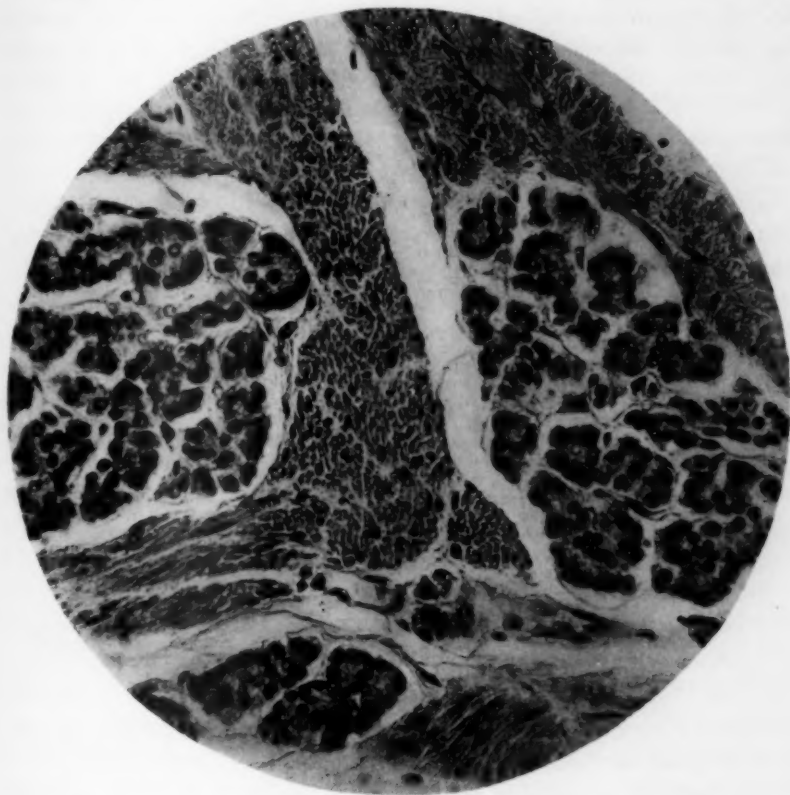


Fig. 5 (case 8).—Lobules of well differentiated pancreas separated by wide bundles of smooth muscle.

CASE 8.—A 46 year old white housewife was admitted to the second medical service with a complaint of diarrhea, fever and abdominal pain of five weeks' duration. Clinically, the course was that of acute enteritis, but no specific diagnosis could be established. She died six days after admission. At postmortem examination four small punched-out ulcers were found in the duodenum just distal to the pylorus. These measured only 2 to 3 mm. in diameter. The wall beneath them was definitely thickened, but no discrete tumor was noted. Microscopically, there was a variety of epithelial formations. A few solid buds of undifferentiated epithelial cells were found. There were many ductlike spaces of varying size and

shape. These were sometimes lined by cuboid cells and sometimes by tall columnar cells with clear cytoplasm. There was much well differentiated pancreatic tissue. These epithelial elements in places seemed to be within a hypertrophied normal muscle wall. Elsewhere there were distinct muscle formations external to the normal muscle coats.

COMMENT

Lesions of the nature of those found in these 8 cases have been described under several different designations. By far the greatest number have been recorded as aberrant pancreas. In order to circumvent the difficulty interposed by the frequent occurrence of undifferentiated

Summary of the Eight Cases Reported

Case; Sex; Age, Yr.	Size, Cm.	Location	Nature of Epithelial Elements	Complications
1 M 18	2 by 2 by 1.5	Antrum of stomach, in submucosa, attached to muscle	Undifferentiated ducts	Pyloric obstruction
2 M 15	2.5 by 2 by 1.5	Meckel's diverticulum, in submucosa	Obscured by infarction—pancreatic tissue	Intussusception of ileum
3 F 53	0.8	Antrum of stomach, in submucosa, attached to muscle	Undifferentiated ducts, pancreas, Brunner's glands	Duodenal ulcer
4 F 58	1.5	Just proximal to pylorus, in muscle wall of stomach	Ducts with cuboid and columnar cells, some Brunner's glands	Ulceration of gastric mucosa and abscesses within tumor
5 F 39	Uncertain about 2 by 1 by 0.5	At pylorus in subserosa	Ducts, pancreas, Brunner's glands	None
6 F 31	1.5	Gallbladder, in subserosa and muscle wall	Ducts lined by tall columnar cells	Chronic cholecystitis and cholelithiasis
7 M 64	1.0	Jejunum, in muscle and subserosa	Ducts only	None
8 F 46	Not observed grossly	Duodenum, in submucosa and muscle	Solid cords of undifferentiated cells, ducts, pancreas	Ulceration of overlying duodenal mucosa

ductlike formations along with the pancreatic tissue, Lauche¹ suggested a subdivision of "incompletely differentiated accessory pancreas." A second popular designation is that of adenomyoma. Strictly speaking this should be applied when only muscle and undifferentiated ductlike structures are present. More rarely the term Brunner's adenoma is used. Thus, of my 8 specimens, no. 2 would be called aberrant pancreas, nos. 1, 4, 6 and 7 adenomyoma and nos. 3, 5 and 8 incompletely differentiated aberrant pancreas. Duodenal gland formations occur only in the mixed group (see table). If these purely morphologic criteria were accepted and adhered to, the confusion would not be so great. However, it is usual to find examples of all these histologic types reported together under any one of the foregoing designations. This in itself indicates

1. Lauche, A.: Virchows Arch. f. path. Anat. **252**:39, 1924.

that many writers have considered them essentially identical, but they have failed to make that point clear or to mention the confusion which occurs in the literature.

A study of the 8 cases together with a review of pertinent publications indicates that the diversity of histologic structure is dependent on degree of differentiation rather than on point of origin or on original cell type. The complicated embryonic mechanisms in the region of the pylorus in connection with the development of the pancreas and the bile ducts probably account for the frequency with which these defects are found in this location (5 of my 8 cases). However, the incidence of the undifferentiated masses seems to be just as great in this region as that of the tumors that differentiate to form pancreas or duodenal glands (3 to 2 in my series). Also, the fact that well differentiated aberrant pancreatic tissue has been described in far removed locations (jejunum, ileum, Meckel's diverticulum, peritoneum, spleen [Danzis²]) indicates that embryonic remnants from any part of the gastrointestinal tract are capable of differentiating into pancreatic tissue. Moreover, the presence of various degrees of differentiation from solid epithelial buds through undifferentiated ductlike spaces to adult pancreatic or duodenal tissue all within the same tumor (cases 3, 5 and 8) is additional evidence that all types may originate from the same remnant. Too little is known concerning the mechanism of normal differentiation to justify any speculation as to why the degree of differentiation in these tumors varies. Embryologically, there occur in the gastrointestinal tract a number of widespread embryonic epithelial buds, most of which normally disappear before birth. Stewart and Taylor³ accept these as the origin for their 4 examples of "adenomyoma." Woolsey and Millzner⁴ considered both accessory pancreas and adenomyoma as heterotopias of the digestive tract of congenital origin, developing from epithelial buds. Branch and Gross⁵ reviewed all suggested origins of aberrant pancreas and concluded that there is little to support any one theory. They concluded that the anomalies arise as the result of congenital aberrations from normal development. Most writers have rejected the suggestion of King and MacCallum⁶ that these masses may originate from normal gastric epithelium as a result of chronic inflammation. In view of this agreement as to the probable origin of these tumors it seems desirable to designate them by a single all-inclusive term based on pathogenesis.

2. Danzis, M.: *Surg., Gynec. & Obst.* **67**:520, 1938.

3. Stewart, M. D., and Taylor, A. L.: *J. Path. & Bact.* **28**:195, 1925.

4. Woolsey, J. H., and Millzner, R. J.: *Arch. Surg.* **16**:583, 1928.

5. Branch, C. D., and Gross, R. E.: *Arch. Surg.* **31**:200, 1935.

6. King, E. S. J., and MacCallum, P.: *Arch. Surg.* **28**:125, 1934

The relationship of the smooth muscle tissue remains unexplained. Is the muscle a secondary proliferation which is in some way stimulated by the misplaced epithelium? Was the initial lesion a malarrangement of muscle which in turn pinched off or deflected the epithelial tissue? All writers have stressed the part of such muscle tissue in those lesions which are designated as adenomyoma. Most authors when discussing aberrant pancreatic tissue have made no comment concerning muscle, but attention is frequently called to it in their microscopic descriptions or photomicrographs. Stewart and Taylor³ mentioned the presence of muscle in a "fully differentiated pancreatic heterotopia" in the jejunum. Some consider it to be the normal muscle which is invaded by the aberrant growth. In 7 of my 8 cases there was a definite increase of muscle tissue. Its occurrence as a fairly discrete nodule extending into the submucosa or the subserosa, together with the unpatterned arrangement of the interlacing bundles, indicates that it was not a portion of the normal musculature invaded by epithelium. On the other hand, the fact that in each case it at some point fused with the normal muscle suggests that it may have originated from normal muscle as a result of some stimulus to proliferation emanating from the misplaced epithelium. I am inclined to believe that this is very probably the correct interpretation. Variation in the relative proportions of muscle and epithelium is marked. If at times this reaches the point at which no muscle is demonstrable, that is quite understandable and is no reason for suspecting a different pathogenesis or for proposing a different name.

The problem of the relation between heterotopia and new growth is a difficult one. Whether such anomalies should be regarded as true neoplasms is problematic. Indeed it is uncertain that they are capable of growth after birth. The development of symptoms in adulthood suggests an increase in size. This, however, is sometimes the result of infection (case 4) or of ulceration of the overlying mucosa (case 8). The term "hamartoma" originally suggested by Albrecht is generally accepted for such tumor-like masses of embryonic origin. It is therefore suggested that this term combined with the descriptive term "myoepithelial" be used to designate this entire group. The designation seems to be all inclusive and indicates the pathogenesis and the component tissue elements.

SUMMARY

Eight cases of benign myoepithelial tumor-like structures of the gastrointestinal tract are reported. Considerations of the pathogenesis and nomenclature of such findings lead to the suggestion that they be designated as myoepithelial hamartoma.

DISTRIBUTION OF AFFECTED NERVE CELLS IN AMYOTONIA CONGENITA (SECOND CASE)

J. LEROY CONEL, PH.D.

BOSTON

In 1938 I¹ presented a case of amyotonia congenita in which the degeneration of nerve cells was in progress, so that it was possible to gain some knowledge of the distribution of the neurons which were affected (case 1). The case presented now, designated as case 2, is likewise one of amyotonia congenita in an active state, but the damage was much less severe than in the former case.

REPORT OF A CASE

A 5½ month old infant, a girl, was brought to the hospital on November 10 because of heavy breathing. Four days later the baby was again brought to the hospital in a choking condition. About an hour previously the mother had given it a feeding and then left it to continue eating alone. When she returned, in about fifteen minutes, she found the baby gasping, blue and obviously choking. She brought it to the hospital immediately. She stated that the baby had always breathed very loudly but that the mental development of the child appeared normal. Examination at the hospital showed that the extremities were somewhat limp and that the infant was rather inactive. Breathing was in shallow gasps at irregular intervals, and soon ceased. The diagnosis was asphyxia, due to aspiration, and amyotonia congenita.

In an attempt to get more information regarding the condition of the muscular weakness in the infant the mother was interviewed, Feb. 3, 1940, and the following data were elicited, based on her memory: The pupillary reactions and ocular movements were normal; there was considerable difficulty in swallowing; the infant was unable to sit up or even to raise its head; the arms and legs were limp and were rarely moved; there was a slight right scoliosis; the infant had a deformity of the chest from birth and always had had difficulty in breathing; the respiration was abdominal in type; in general, the infant showed little motor activity; its mental development seemed normal to the mother. There is a 9 year old sibling living and well.

MATERIAL AND METHODS

At autopsy the entire brain and spinal cord were removed. A few dorsal root ganglions in the lumbar and sacral regions and a piece of the thoracic sympathetic chain were taken. Small specimens of the quadriceps femoris, psoas major, rectus abdominis, diaphragm, intercostal, triceps and biceps brachii muscles were saved.

From the Department of Anatomy, Boston University School of Medicine, and the Massachusetts Memorial Hospitals, and the Department of Pathology, Harvard Medical School, and the Children's Hospital and the Infants' Hospital.

1. Conel, J. L.: Arch. Neurol. & Psychiat. **40**:337, 1938.

All the nerve tissue was fixed in 10 per cent neutral formaldehyde solution. The pieces of muscle were fixed in 10 per cent formaldehyde solution and in Zenker's fluid. All the tissues were embedded in paraffin. The entire spinal cord and brain stem were cut in sections 20 microns thick. About every eleventh and twelfth section were stained by Spielmeyer's myelin method and a modification of the Cajal silver method, respectively. All the other sections were stained with cresyl violet and eosin. Sections of the muscle tissue were stained with hematoxylin and eosin. The cortex of the right cerebral hemisphere was cut in small blocks and embedded in paraffin. Sections were cut 25 microns thick and stained with cresyl violet. A section from each block was stained by the Spielmeyer and Cajal methods.

Sections through the entire spinal cord and brain stem of an infant 6 months old have been used as a control. The material both in case 2 and in the control case is well preserved, and the cells are in exceptionally good condition.

OBSERVATIONS ON CELLS

Spinal Cord.—(a) Lower Sacral Region: In each section through the clusters of cells where they are the most numerous there are about 25 normal cells in the ventral horn of gray matter on each side of the cord. These are small and medium-sized multipolar cells containing large, darkly stained Nissl bodies of normal appearance. In the ventral horn on each side of a normal spinal cord in this region from 75 to 85 normal cells are present. In sixty serial sections through this region of the cord 10 affected cells have been counted, 5 in the early and 5 in the intermediate stage, about equally distributed between the two sides of the cord.

In the lower sacral dorsal root ganglions most of the nerve cells are of normal appearance and have well stained nuclei and Nissl bodies. In each section through the largest part of a ganglion there are about 20 to 25 slightly shrunken cells with darkly stained cytoplasm in which Nissl bodies cannot be distinguished. Also, in each section there are from 15 to 25 clusters of small, darkly stained nuclei, each cluster occupying the capsule of a neuron, but no traces of the nerve cell are apparent (fig. 1). These clusters may be the residual nodules of Nageotte,² which he believed consisted of neuroglial phagocytic cells. Whether they are or not is difficult to determine from his description, and he did not illustrate them. Perhaps each cluster represents the final stage in the removal of the remnants of an affected nerve cell. Affected cells typical of amyotonia congenita are also present in the dorsal root ganglions. In sixty serial sections through a large ganglion on either side of the cord there are about 30 affected cells, 25 in the intermediate and 5 in the late stage. One of these cells is shown in figure 2 A.

(b) Middle of the Lumbar Enlargement: In this region the ventral column of gray matter is smaller than normal. In each section passing through the clusters of cells in the ventral horn normal multipolar cells

2. Nageotte, J.: Anat. Anz. **31**:225, 1907.

number about 40 on each side of the cord. In a normal cord there are from 75 to 85 normal cells on each side. There has been, therefore, considerable reduction in the number of normal cells. Two or 3 shrunken cells are present in each section. In sixty serial sections 6 typical affected cells are present in the ventral horn, 3 in the early and 3 in the intermediate stage, equally divided between the two sides.

The cells in Clarke's column (nucleus dorsalis) are normal in size and appearance, and in the clusters they number about 20 to 24 to the section on each side. This is about the number in a normal cord. In

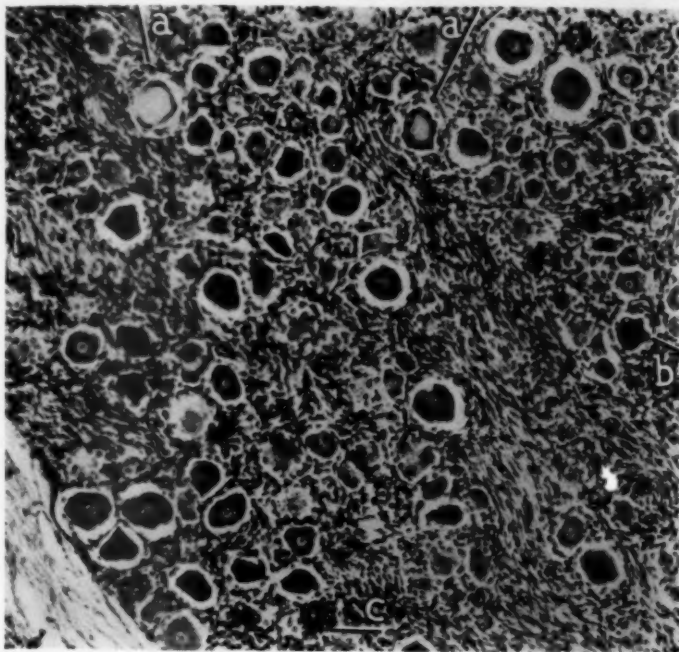


Fig. 1.—Photomicrograph of a portion of a dorsal root ganglion showing normal cells, 2 affected cells (*a*), a few shrunken, darkly stained cells (*b*) and some of the clusters of small, darkly stained nuclei (*c*).

sixty serial sections 2 affected cells, both in the intermediate stage, were found in this column, one on the right, the other on the left side (fig. 2 *B*).

(*c*) Upper Lumbar Region: The gray substance in the ventral column is smaller in size than normal. Not more than 6 normal cells have been counted in any section which passes through a cluster of cells in the ventral horn, and 4 to 6 small, shrunken cells are present in each section. Many sections contain only 1 or 2 normal cells. In a section through a cluster of cells in a normal cord in this region there are about 20 small normal cells in the medial part of the ventral horn and approxi-

mately 50 to 55 medium-sized and large normal cells in the lateral part. In sixty serial sections only 2 affected cells, both in the intermediate stage, are present in the ventral column.

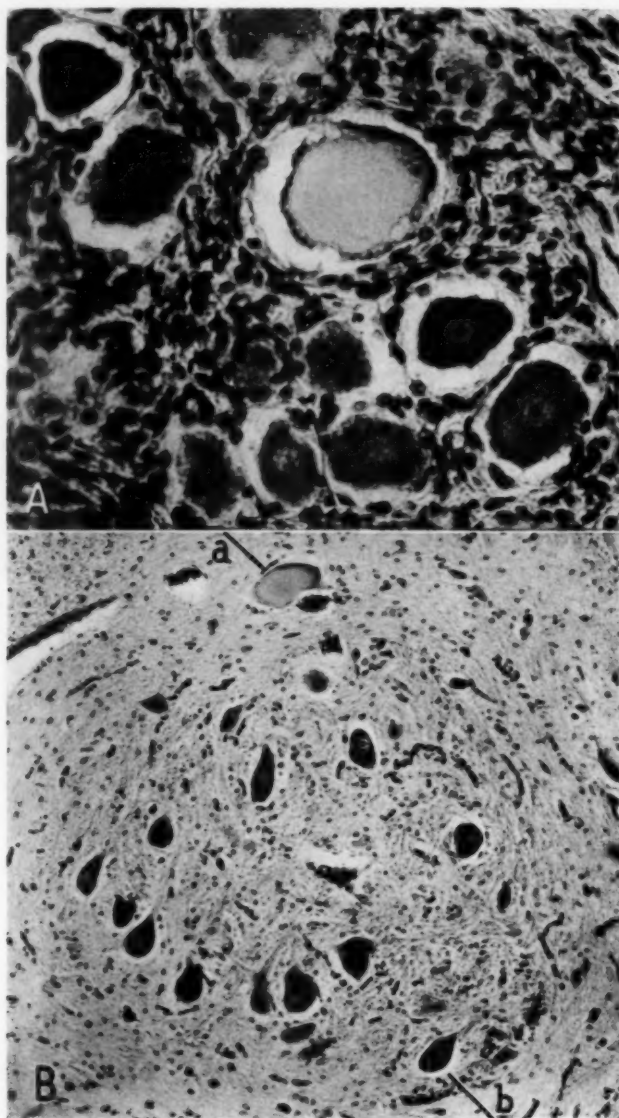


Fig. 2.—*A*, affected cell in the intermediate stage of degeneration in a dorsal root ganglion. *B*, affected cell (*a*) in the intermediate stage of degeneration in Clarke's column. The nucleus of a normal cell (*b*) is always near the periphery of the cell body.

The cells in Clarke's column in this region are well rounded and normal in appearance and are of about the normal number. In sixty serial sections 3 affected cells, all in the intermediate stage, were found, 1 in the right column and 2 in the left.

Most of the cells in the dorsal root ganglions in this region are of normal size and appearance. In each section through the central part of a ganglion, however, from 10 to 12 slightly shrunken, darkly stained cells are present. There are from 10 to 15 residual nodules in each section. In sixty serial sections 6 affected cells, all in the intermediate stage, are present.

(d) *Midthoracic Region*: The anterior column of gray matter is smaller and the motor cells are fewer than in the normal. In sections passing through the clusters of cells there are from 6 to 8 small, shrunken cells and from 1 to 4 normal cells. Many sections through this region show no normal cells in the ventral column, but there are usually a few shrunken ones. In this region of the normal cord there are about 20 small and medium-sized normal cells on each side in each section which passes through a cluster of cells. In sixty serial sections only 1 affected cell has been found in the anterior column of gray substance. In these same sections 14 affected cells are present in Clarke's column, 5 in the early and 9 in the intermediate stage. Of these cells, 6 are in the left column and 8 are in the right. No dorsal root ganglions were obtained from this region. No affected cells have been seen in the intermediolateral column, and the cells are normal in number and appearance.

(e) *Upper Thoracic Region*: The gray substance in the anterior column is reduced in size, and the motor cells are much reduced in number as compared with the normal. No more than 3 normal cells and 10 small, shrunken cells have been counted in the anterior column in any one section. In many sections there are only 2 or 3 small, shrunken cells and no normal ones. There are about 20 small and medium-sized cells in the ventral horn on each side in a section which passes through a cluster of cells. In sixty serial sections through this region of the cord in case 2 only 3 affected cells have been seen in the ventral column, 1 in the early stage, on the right side, and 2 in the intermediate stage, on the left side. In the same series of sections there are 2 affected cells in Clarke's column, both in the early stage. There are no affected cells in the intermediolateral column, and the cells are normal in number and appearance.

(f) *Midregion of the Cervical Enlargement*: Considerable reduction has occurred both in the quantity of gray matter and in the number of cells in the ventral column. In sections where the cells are the most numerous they number about 15 normal and 4 or 5 small, shrunken ones in the lateral part of the column and about 4 to 6 normal and

12 to 15 small, shrunken ones in the medial part. In all sections the cells in the lateral part of the anterior column are in much better condition than those in the medial part; many sections have no normal cells in the medial part. In a section through this region in a normal cord there are about 15 small and medium-sized cells in the medial part of the ventral column and 75 medium-sized and large cells in the lateral part. Only 3 affected cells, all in the intermediate stage, have been counted in the ventral horn in sixty serial sections through this region of the cord in case 2. Clarke's column extends into this region, and the cells number 7 to 10 on each side in each section in the clusters and 2 to 4 between the clusters. The cells are normal in appearance. Only 1 affected cell has been found in sixty serial sections, and that is in the intermediate stage. No dorsal root ganglions were taken from this region.

(g) Upper Part of Cervical Enlargement: There are about 12 to 15 small, medium-sized and large normal cells in the ventral horn on each side in each section which passes through clusters of cells. These cells in a normal cord number about 20 to 25 in each section through the clusters; therefore there has been some reduction. There are a few small, shrunken cells in each section. In sixty serial sections through this region 14 affected cells are present, 6 in the early and 8 in the intermediate stage, about evenly divided between the two sides.

(h) Upper Part of Cervical Region: The gray matter does not appear to be reduced in size, and normal cells are but slightly decreased in number. In each section which passes through a cluster of cells in the ventral horn there are from 12 to 15 normal small, medium and large cells and about 25 to 30 small, shrunken cells on each side of the cord. Some of the shrunken cells are elongated and appear to have been large cells. Many sections show but 1 or 2 normal cells. In the normal cord there are about 15 to 18 small, medium and large normal cells and 25 to 30 small cells with very fine, lightly stained Nissl granules. In sixty serial sections through the cord in this region in case 2 there are only 5 affected cells, 2 of which are in the early and 3 in the intermediate stage. These affected cells are located in the medial part of the ventral column. No affected cells are present among the cells of the accessory nerve in the lateral part of the gray substance. Most of the cells in this nucleus are normal in appearance, but there are some shrunken ones in every section.

Brain Stem.—In sixty sections through the region of the decussation of the pyramids only 3 affected cells are present in the continuation of the ventral column rostrally, here known as the nucleus supraspinalis. The affected cells are in the intermediate stage. Two are on the left, and the other is on the right side. Normal cells are scattered along the entire extent of this nucleus as far rostrally as the nucleus of the hypoglossal nerve. In many sections there are only 1 or 2 normal cells, but

as many as 10 have been counted in sections which pass through clusters of cells. A few shrunken cells are present in each section.

Most of the cells in the nucleus of the accessory nerve are normal in appearance, but a few in each section are shrunken. No affected cells have been found in this nucleus.

The sections in this region pass through the caudal end of the nuclei gracilis and cuneatus. Very few of the cells in the nucleus gracilis are normal in appearance. All others are shrunken, small and darkly stained. The opposite is true of the nucleus cuneatus, however. No affected cells have been seen in either of these nuclei.

Affected cells become more numerous in the nucleus supraspinalis the nearer the nucleus of the hypoglossal nerve is approached. There is a considerable reduction in the number of normal cells in the hypoglossal nucleus as compared with the normal. In sections passing through the nucleus where the cells are the most numerous from 25 to 28 normal cells can be counted on each side, whereas in the control specimen 55 normal cells are present on each side in a section through the middle of this nucleus. In sixty serial sections (20 microns thick) through the hypoglossal nucleus 115 affected cells are present, 36 in the early and 79 in the late stage. Of the cells in the early stage 17 are on the right side and 19 are on the left, and of the cells in the intermediate stage 36 are on the right and 43 are on the left side.

Only 7 affected cells are present in the entire extent of the nucleus ambiguus, 3 in the early and 4 in the intermediate stage; 3 of the affected cells are on the left side and 4 are on the right.

In the nucleus of the facial nerve there are 30 affected cells, of which 8 are in the early, 21 in the intermediate and 1 in the late stage. The affected cells are evenly divided between the right and left sides. The nucleus of the facial nerve is linked to the motor nucleus of the trigeminal nerve by a strand of motor cells, numbering from 1 to 4 in a section. Among these cells there are 9 affected ones, 5 in the early and 4 in the intermediate stage; 6 of these cells are on the left side and 3 are on the right.

Only 1 affected cell has been found among the multipolar motor cells in the reticular formation, and 1 affected cell is present in the lateral vestibular nucleus. The cells in this nucleus are large multipolar ones with large nuclei. A few cells in both the formatio reticularis and the lateral vestibular nucleus are shrunken.

There are 13 affected cells in the nucleus of the abducens nerve, 12 in the intermediate and 1 in the early stage. Of these cells 9 are on the left and 4 are on the right side. A few of the cells in these nuclei in each section are shrunken.

No affected cells are present anywhere in the motor nucleus of the trigeminal nerve.

In the nucleus of the trochlear nerve there are 22 affected cells in twenty-five serial sections. Of these cells 1 is in the early, 13 are in the intermediate and 8 are in the late stage. Thirteen of the cells are on the right side and 9 are on the left.

In thirty-six sections through the nucleus of the oculomotor nerve there are 55 affected cells, 1 in the early, 33 in the intermediate and 21 in the late stage. Of these cells, 30 are on the right side and 25 are on the left. The cells do not seem to be reduced in number as compared with the control.

Careful search has failed to reveal any affected cells anywhere in the medulla, pons or midbrain other than in the nuclei mentioned. Also, no affected cells have been found in the cortex or nuclei of the cerebellum.

Almost every low power field in the lateral nucleus of the thalamus contains from 1 to 6 affected cells in the intermediate and late stages. No affected cells have been observed in any other part of the thalamus or in any part of the basal nuclei.

Cerebral Cortex.—No affected cells have been found in the anterior central gyrus.

OBSERVATIONS ON MYELIN

Cord.—There is considerable loss of myelin in the ventral roots in the sacral region, but a few fibers still bear some myelin. Both sides are about the same. Some of the fibers extending from the ventral column to the periphery of the cord are stained in the Spielmeyer sections. No diminution in the amount of myelin is evident in the dorsal roots. There has been no appreciable loss of myelin within the cord except in the ventral horn.

The lumbar region is in about the same condition in respect to degeneration of myelin as the sacral region, and the midthoracic region is about the same as the sacral and lumbar regions.

The ventral roots in the upper thoracic region are decidedly darker than those in the regions below this level.

Many more myelinated fibers are present in the ventral roots in the midregion of the cervical enlargement than in the upper thoracic region, but these roots do not contain as much myelin as the dorsal roots.

In the upper part of the cervical enlargement the nerve roots are unfortunately not present. There are about as many darkly stained rootlets extending from the ventral horn to the periphery of the cord as are present in the midregion of the cervical enlargement.

No nerve roots are present in the upper cervical region. Rootlets in the cord are in about the same condition as those in the upper part of the cervical enlargement.

The rootlets of the hypoglossal nerve which are still clinging to the medulla contain many lightly stained and unstained fibers.

The rootlets of the accessory nerve contain some unstained fibers. The roots of the vagus and glossopharyngeal nerves are quite darkly stained and do not have any unstained fibers in them.

The rootlets of the facial nerve are fairly darkly stained, but there are some lightly stained and unstained fibers in them.

There are no rootlets of the abducens nerve remaining attached to the brain stem.

The roots of the trigeminal nerve are quite darkly stained and contain no unstained fibers.

A few unstained and lightly stained fibers are present in the roots of the oculomotor nerve.

The pyramidal tracts appear to be normally stained.

Muscles.—The sections of the specimen from the quadriceps femoris muscle contain few normal muscle fibers. There are many of the small fibers which have been described by Foot³ and other authors as characteristic in cases of amyotonia. These fibers are thrown into short wavelike folds throughout their entire length. The small diameter and the folding have brought the nuclei close together and thereby caused them to seem more numerous than in the normal fibers, which are always straight. Some of the small fibers have fairly distinct transverse and longitudinal striations. In others the myofibrils show clearly, but the transverse markings are very faint or entirely lacking.

In the sections of the psoas major muscle large normal fibers are much more numerous than in the quadriceps femoris. There are many small affected fibers scattered through the specimen singly and in groups.

The rectus abdominis muscle shows a very large number of small affected fibers and but few large normal ones.

In the intercostal muscles normal fibers are present in less quantity in proportion to affected fibers than in any other muscles examined. Very few normal fibers are seen in each section.

The diaphragm contains a great many small affected fibers, but normal ones are much more numerous than in the intercostal muscles.

The specimens of the two muscles taken from the upper extremity show that the triceps has suffered more damage than the biceps. Normal fibers are present in greater proportion to affected ones in the biceps than in the triceps, and normal fibers are much more numerous in the triceps than in any of the other muscles examined. The small affected fibers are numerous in these two muscles, however, and are arranged principally in groups of one or two dozen fibers interspersed here and there among bundles of large normal fibers.

Longitudinal sections through the tongue show that the muscle fibers are in good condition. No typical affected fibers are present. Here and there, however, are seen a few small fibers which are slightly wavy.

3. Foot, N. C.: *Am. J. Dis. Child.* 5:359, 1913.

COMMENT

There is some edema in all parts of the spinal cord and brain, but not more than is often present in these structures after autopsy. Nowhere in the nervous system are there evidences of any inflammatory process.

Affected cells are present in the following regions only:

- Ventral column of the spinal cord
- Clarke's column
- Spinal ganglions
- Nucleus supraspinalis
- Nucleus ambiguus
- Nucleus of the hypoglossal nerve
- Formatio reticularis
- Lateral vestibular nucleus
- Nucleus of the facial nerve
- Nucleus of the abducens nerve
- Nucleus of the trochlear nerve
- Nucleus of the oculomotor nerve
- Lateral nucleus of the thalamus

Most of the affected cells are in the intermediate stage of degeneration. As compared with the control, there has been considerable reduction in the number of cells throughout the spinal cord and in the nucleus of the hypoglossal nerve. Presumably this decrease in the quantity of cells is due to complete degeneration of the cells. A rough estimate of the extent of such loss of cells is obtained by counting the normal cells still remaining in sections where the cells are the most numerous and comparing this number with the number of cells in a corresponding section in the control specimen. By using this method the percentages of loss of nerve cells in the ventral column in various regions of the spinal cord and in the hypoglossal nucleus are estimated as follows: lower sacral region, 70; middle of the lumbar enlargement, 53; upper end of the lumbar enlargement, 90; thoracic region, 85; middle of the cervical enlargement, 77; upper part of the cervical enlargement, 40; upper cervical region, 17; nucleus of the hypoglossal nerve, 50. No appreciable loss has occurred in the nucleus ambiguus, the formatio reticularis, the nuclei of the facial, abducens, trochlear or oculomotor nerves or the lateral nucleus of the thalamus. The extent of loss of nerve cells in the ventral column of gray matter in the spinal cord corresponds fairly closely with the degree of degeneration of myelin in the ventral roots and with the amount of damage to muscles as estimated by the number of the small muscle fibers. Unfortunately, specimens of muscles were saved from only a few regions; therefore this correlation cannot be pursued far. The roots of the hypoglossal,

accessory, facial and oculomotor nerves contain some lightly stained and unstained fibers, indicating a slight loss of myelin.

Spinal ganglions were saved from the sacral and lumbar regions only. Typical affected cells, darkly stained, shrunken cells and residual nodules are present in all these ganglions. In each section through some ganglions there are also a few empty capsules. Even if all four of these abnormalities are accepted as evidence of injury to the nerve cells, the amount of damage is not nearly as great as that in the corresponding regions of the ventral column of gray matter in the spinal cord. With the exceptions noted the cells in the ganglions appear to be normal in all respects. All the typical affected cells are large ones. Should the injury to the ganglion cell be interpreted as the result of degeneration of proprioceptive fibers in the muscles? The number of cells that have been damaged is not sufficient to cause any apparent loss of myelin in either the dorsal roots or the peripheral nerves attached to the ganglions. No loss of myelin is evident in the dorsal funiculus. There are no typical affected cells in the nuclei gracilis or cuneatus, but many of the cells in the former and a few in the latter are considerably shrunken. Shrunken cells are not to be considered too seriously, however, as they can be seen in all brains and spinal cords at autopsy, and the shrinking may be caused by postmortem changes.

A few typical affected cells are present in Clarke's column throughout its entire extent. There is no reduction in the number of cells. Excepting occasional shrunken ones, all the cells in this column are in good condition. The significance of the presence of affected cells in the spinal ganglions, in Clarke's column and in the thalamus is not known. Several interpretations are possible on the basis of what is already known, but the real explanation is still unrevealed. If the phenomenon of *réaction au distance* is invoked, several questions arise, among which are that as to the source of the reaction and that as to the reason for the escape from injury of cells in the nuclei gracilis and cuneatus, in the red nucleus, in the tectum mesencephali and in the olive, all of which have direct contact with the cells in either the spinal ganglions or the ventral column of the spinal cord. The cerebellum is once removed from direct contact with the spinal ganglions by way of the cells in Clarke's column, and whereas affected cells are present in the latter, not any were found in the cerebellum.

In cases 1 and 2, reported in this paper, are found the most varied distribution of affected cells yet recorded for amyotonia congenita. In either one or the other or in both of these cases there is reported for the first time the presence of typical affected cells among the Betz cells in the gyrus centralis anterior, in the thalamus, in the nuclei of the oculomotor, trochlear and facial nerves, in the lateral vestibular nucleus

and in the dorsal root ganglions. Spiller⁴ noted a decrease in the number of Betz cells in the paracentral lobule but did not report the presence of any affected cells. Foot⁵ is the only other author who observed affected cells in the nucleus supraspinalis and in the formatio reticularis. Kaumheimer⁶ is the only other writer who found typical affected cells in Clarke's column. All other investigators who have mentioned the diaphragm have reported it to be normal, but in case 2 it contains many affected fibers.

The character and distribution of the affected neurons in the spinal cord and brain are pathognomonic of amyotonia congenita. The gradual degenerative changes in the affected nerve cells reported in cases 1 and 2 and the loss of myelin indicate that the cells involved had been normal cells whose development had been completed even to the extent of the formation of myelin on their axons.

An extensive bibliography for amyotonia congenita and Werdnig-Hoffmann disease is listed by Grinker.⁶

SUMMARY

The brain, spinal cord and dorsal root ganglions of an infant 5½ months of age who presented clinical evidences of amyotonia congenita were removed at autopsy. Sections prepared from all parts of these structures were carefully examined for evidences of pathologic changes. Such evidences were found only in nerve cells and consisted of gradual degeneration of the cell body, a condition which is easily recognized and has been fully described in a previous paper. The affected nerve cells were previously definitive normal cells, at least some of which had myelinated axons. The degeneration of the cell body was accompanied by a corresponding loss of myelin from the axons. In either case 1 or 2 or in both, affected cells were found among the Betz cells in the gyrus frontalis anterior, in the lateral nucleus of the thalamus, in the somatic efferent nuclei of the oculomotor, trochlear and abducens nerve, in the branchial (special visceral) efferent nuclei of the facial, glossopharyngeal, vagus and accessory nerves (nucleus ambiguus), in the lateral vestibular nucleus, in the somatic motor nucleus of the hypoglossal nerve, among the motor cells in the formatio reticularis and nucleus supraspinalis, in Clarke's column, among the somatic efferent cells in the anterior column of gray matter in the spinal cord and in the dorsal root ganglions.

Affected neurons are reported here for the first time in the nucleus of the trochlear nerve, in the lateral vestibular (Deiters') nucleus and in the spinal ganglions.

4. Spiller, W.: Univ. Pennsylvania M. Bull. **17**:342, 1905.

5. Kaumheimer, L.: Jahrb. f. Kinderh. **78**:170, 1913.

6. Grinker, R. R.: Arch. Neurol. & Psychiat. **18**:982, 1927.

SOME EFFECTS OF CHRONIC ALCOHOL POISONING IN RABBITS

CHARLES L. CONNOR, M.D.

SAN FRANCISCO

Many experiments having to do with the acute effects of alcohol on man and lower animals have been reported, and some results of continued alcohol poisoning are on record, mostly concerned with attempts to produce cirrhosis of the liver. The physiologic effects on man have been shown not to enter considerably into the intimate metabolism of muscle, nor to be concerned with the chemical reactions and interreactions of proteins, carbohydrates and fats. Much of the work on the latter phase of the subject has been done by Carpenter,¹ who indicated that alcohol, by furnishing calories, may perhaps take the place of fat but not that of carbohydrate or that of protein, and by Bollman and Mann,² who discussed the physiologic and pathologic changes in the liver under a large variety of conditions, including the effects of toxic agents. Reports of work on the more chronic anatomic effects were reviewed and the results abstracted by Moon.³

The development in depancreatized dogs⁴ and in patients with diabetes of cirrhosis of the liver of a type indistinguishable from that found in chronic alcoholism led to an inquiry into the nutrition and possible biochemical states of patients addicted to the consumption of alcohol, and into the histogenesis of alcoholic cirrhosis of the liver.⁵ From this and the reports of Higgins,⁶ Himwich and his co-workers,⁷ and many others on the respiratory quotient and the accumulation of sugar, lactic acid and carbon dioxide in the blood, on the obvious lack of proper respiration of tissue and on chronic fatty infiltration of the liver, the rather broad statement was made that the two conditions, diabetes and alcoholism, were in some respects much alike. It was suggested that in alcoholism there might be internal starvation, as there

From the Division of Pathology, University of California Medical School.

This work was supported by the Christine Breon Fund for Medical Research.

1. Carpenter, T. M.: *Scient. Monthly* **45**:5, 1937.

2. Bollman, J. L., and Mann, F. C.: *Ergebn. d. Physiol.* **38**:445, 1936.

3. Moon, V. H.: *Arch. Path.* **18**:381, 1934.

4. Chaikoff, I. L.; Connor, C. L., and Biskind, G. R.: *Am. J. Path.* **14**:101, 1938.

5. Connor, C. L.: *Am. J. Path.* **14**:347, 1938.

6. Higgins, H. L.: *J. Pharmacol. & Exper. Therap.* **9**:441, 1917.

7. Himwich, H. E.; Nahum, L. H.; Rakiety, N.; Fazikas, J. F.; Du Bois, D., and Gildea, E. F.: *J. A. M. A.* **100**:651, 1933.

is in diabetes, due to the inability of the tissues to utilize sugar and other food substances. In addition, in alcoholism in man it was found that actual or partial starvation frequently occurred, and that when food was eaten during the periods of severe alcoholism it was more likely to consist largely of proteins and fats than of carbohydrates.

In order to investigate further aspects of this problem, experiments were set up in which rabbits and monkeys were maintained on diets containing larger amounts of protein and fat and less carbohydrate than these animals are accustomed to eat. The experiments with rabbits have continued long enough, so that now some of the results produced in this group may be reported.

METHODS

Two diets were tried for the rabbits: a casein mixture and a soybean diet. The casein diet consisted of crude casein 50, cottonseed oil 25, ground filter paper 25, salt mixture (Mendel) 1 and vitamins in the form of Caratol (A & D), and Galen B (B complex). The rabbits received lettuce leaves in moderate amounts twice a week, and at irregular intervals, lasting from several days to a week, it was necessary to place them on the usual normal diet of greens, rolled barley and alfalfa. Later the soybean diet was substituted for this: cracked soybeans 100 and salts 1, with vitamins as before, but no greens. The rabbits readily became accustomed to this diet, and control animals on this diet alone gained remarkably in weight. The soybean diet is calculated (after Horvath, cited by Carpenter and Lee ⁸) to contain approximately 40 per cent protein, 18 per cent fat and 6 per cent available carbohydrate. It contains lecithin up to 3.82 per cent. The animals ate from 75 to 125 Gm. daily.

Alcohol was given in 20 per cent concentration by stomach tube to all the treated animals, beginning with a daily dose of 20 to 50 cc. and increasing the dose as tolerance was established, in some instances over 100 cc. at a time. The tolerance varies in different animals of the same species, so that an amount per kilogram of body weight does not express a relative effect. The best indication of toleration of a given dosage on the part of a treated animal is gain in weight; conversely, overdosage causes continuous loss in weight. It will be seen later that with time a tolerated dose became intolerable; at this time the observed prolonged coma and loss of appetite signaled a loss of tolerance, and in many instances the alcohol was stopped at this time or the dose reduced. For these various reasons the daily doses of alcohol were varied in order to keep the animals alive. I was not interested in the acute toxic effects of alcohol but in establishing a metabolic state which was tolerable but far from normal, for as long a period as possible.

RESULTS

The observations on 51 of 64 rabbits are included in this report, 13 animals having been discarded because of incidental infections or other factors which might influence the validity of the results. Complete autopsies were done on all animals which died or were killed. I believe

⁸ Carpenter, T. M., and Lee, R. C.: *J. Pharmacol. & Exper. Therap.* 60: 264, 1937.

that all of the findings recorded here as positive were actually the result of the experimental conditions imposed. Thirteen rabbits were on the casein diet for periods varying from eight to one hundred and twenty-nine days. Thirty-eight were on the soybean diet from nineteen to three hundred and four days. In the groups on the casein mixture the experiment was complicated by an element of starvation, as the animals did not eat this diet well. This is reflected in the shorter period during which they could be kept alive, also in the greater and more rapid loss of weight. Even though living on the average a much shorter time, the casein group had lost an average of 33 per cent of their weight when they died, compared with an average loss of 16 per cent in the longer treated but better fed soybean groups. Nevertheless, two of the

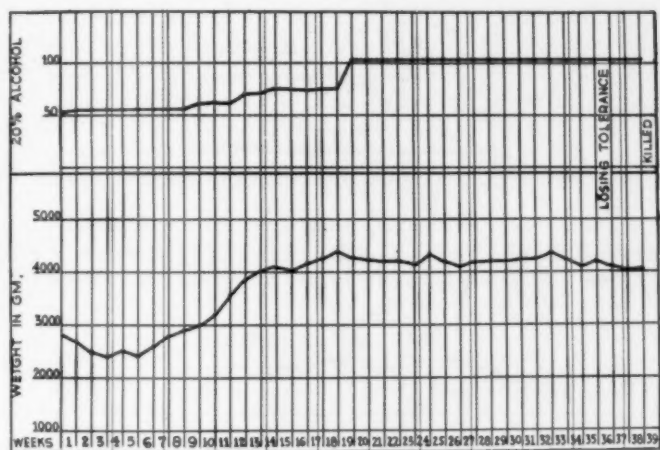


Fig. 1.—Rabbit 28, treated from Sept. 20, 1938 to June 20, 1939. This rabbit tolerated alcohol well but finally lost its tolerance, even though the experimental conditions were not changed. The immediate loss of weight is characteristic, followed in those animals which tolerated the dose given by a gain in weight until toward the last.

changes to be described, fatty infiltration and liver cell atrophy, did not differ qualitatively in the two groups; the starvation accelerated the development of these changes in the casein group. Because of the longer life of the rabbits on the soybean diet, fibrosis developed more extensively and more commonly in this group. With this exception being kept in mind, the two groups may be discussed together.

When the administration of alcohol had been started, the effect on nutrition could be observed by an immediate loss in weight, generally followed by recovery and a gain in weight when the amount of alcohol was not increased too rapidly (fig. 1, rabbit 28). A continuing loss of weight was produced by continuing the administration of a poorly

tolerated dose of alcohol or by increasing the dose so rapidly that tolerance could not be established (fig. 2, rabbit 5). In some animals this rapid decline was undoubtedly due to lack of intake of food; in others it occurred even though an abundance of food had been eaten; it occurred in all the animals in the course of time, after having gained weight or merely maintained their weight over many weeks, even though the dose of alcohol may actually have been decreased when loss of tolerance had been observed. Rabbit 25 (fig. 3) was killed when it became obvious that it was going to die. From experience with others which died (e. g., rabbit 30, fig. 4) this final loss of tolerance and

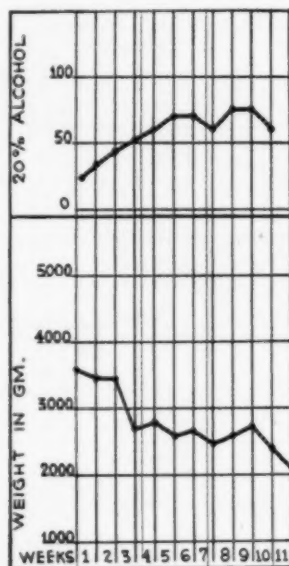


Fig. 2.—Rabbit 5, treated from March 3 to May 21, 1938. A continued loss of weight was produced by what was for this animal an excessive amount of alcohol. The rabbit died.

weight presaged the end, for it indicated that the liver (and possibly other organs) was so badly damaged that death could not be prevented by stopping the alcohol and feeding a normal or a high carbohydrate diet. This did not happen to control animals on the same diets. Casein-fed controls did not lose much weight, but that diet was soon abandoned for the far better soybean diet. Six control rabbits have been on soybeans for over eighteen months, have gained enormously in weight (1 weighs 4,400 Gm.) and have maintained their weight. It seems necessary to note that whether the animals lost weight because they did not eat or could not assimilate what they ate or could not utilize what they assimilated, the factor in the experiment which caused this change from the normal was the introduction of alcohol.

Four effects were produced on the liver: (1) fatty infiltration; (2) atrophy, primary or following fatty infiltration; (3) cellular degeneration of varying degrees, sometimes of a slow coagulative hyaline type,

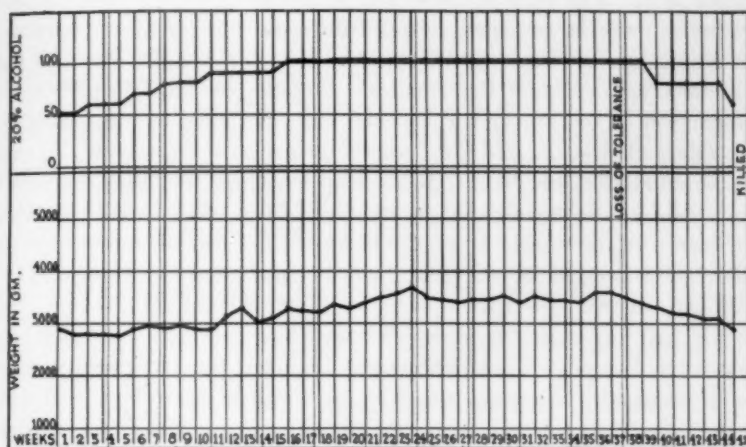


Fig. 3.—Rabbit 25, treated from Aug. 26, 1938 to June 30, 1939. Note the characteristic decline in weight after the loss of alcohol tolerance even though the dose of alcohol was decreased. The animal would have died.

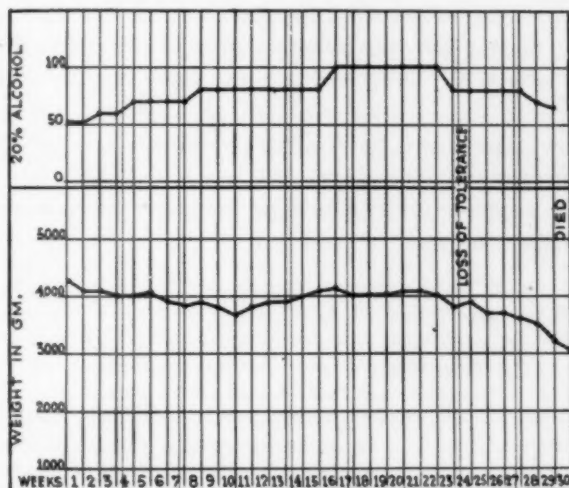


Fig. 4.—Rabbit 30, treated from Nov. 2, 1938 to June 16, 1939. Cirrhosis of the liver developed. The animal barely tolerated the dose of alcohol given. This indicates the narrow margin which it seems necessary to maintain between the dose of alcohol and the nutrition to secure maximal results.

sometimes rapidly necrotizing, and (4) fibrosis in variable amounts with perilobular or portal distribution. These changes were frequently mixed, forming a rather large number of combinations: fatty infiltration with necrosis or hyaline degeneration or fibrosis; cell atrophy with or without areas of fatty infiltration and with or without fibrosis. Grossly, the livers were larger than normal and pale and fatty, or normal in size and color, or markedly atrophied. The top weight was 160 Gm., in a 4,000 Gm. rabbit; the smallest, 34 Gm., in a 2,100 Gm. rabbit. Comparison of the average weight, 80 Gm., with the average weight of the rabbits at death, 2,522 Gm., yields no useful information. A rabbit may be emaciated and have a large fatty liver; another may have lost a great deal of weight and have an atrophied liver. For instance, rabbit 9 had decreased in weight from 2,700 to 1,925 Gm. in twelve days and had a liver weight of 42 Gm.; rabbit 10 had gone from 2,700 to 1,900 Gm. in twenty-six days and had a liver weight of 130 Gm. Both animals had been on the same diet and had received the same daily amount of alcohol. These examples illustrate the irregularity of the results obtained. Preceding death in all instances there had been a loss of tolerance to alcohol and always a loss of weight. There was evidence in those dying, early or late, that some previous fatty infiltration had occurred in most of them. Four rabbits killed relatively early had fatty infiltration; 3 with atrophy had residual areas of fatty infiltration, and many of the 18 with more or less fatty infiltration (as distinguished from those in which atrophy predominated) had areas in which some liver columns were undergoing atrophy.

In spite of this irregularity of results, of the mathematical combination of effects, the variations in time which it took to produce the same effects or the identical time to produce different effects, the results of the experiments have formed a pattern from which plans for future experiments may be drawn. Only a few animal histories will be given to represent the extreme and the average effects, as nearly as an average can be estimated.

Rabbit 3.—Casein diet; lived forty-two days; killed when moribund. Weight at start 3,200 Gm., at death 2,100 Gm. Received 25 cc. of 20 per cent alcohol by stomach tube for six days, 45 cc. for five days, 50 cc. for six days, 60 cc. for four days and 70 cc. for nine days, a total of 1,855 cc. (371 cc. of absolute alcohol). Autopsy report: The rabbit is emaciated, with little fat present. The lungs are somewhat edematous, the kidneys pale, the liver very small (weight 34 Gm.). Microscopic observations: All the organs are normal except the kidneys and the liver. The kidneys show degeneration of convoluted tubules, the cells are swollen, and some are desquamated into the lumen. Some cells contain large fat vacuoles. The liver is entirely devoid of glycogen and fat. The cells are markedly atrophied, forming shrunken distorted columns, and the sinusoids are dilated but empty. (The following note was made at the time this liver was described: "The cells of this liver and of one previously described, rabbit 9, both from very small livers, bear

a close resemblance to the small cells described by McNider as regenerated resistant cells after uranium, chloroform and alcohol poisoning [McNider, W. deB., J. Pharmacol. & Exper. Therap. 56:359, 1936]. But these cells are not regenerated cells; they show simply a high degree of atrophy").

Rabbit 10.—Casein diet; lived twenty-six days; died of alcohol poisoning. Weight at start 2,700 Gm., at death 1,900 Gm. Received 40 cc. of 20 per cent alcohol by stomach tube for seven days, 50 cc. for nine days and 40 cc. again for seven days, a total of 1,010 cc., or 202 cc. of absolute alcohol. The animal was found dead three days after the administration of alcohol was stopped. Autopsy report: The rabbit is emaciated, with almost no depot of fat. The lungs are red and edematous but contain some air throughout; the kidneys weigh 19 Gm. together; the liver is large and pale and looks fatty; the weight is 130 Gm. No infection, no other gross lesion is apparent. Microscopic observations: The lungs show congestion and edema only; the kidneys, besides some postmortem degeneration, show slight fatty infiltration of the epithelium of the convoluted tubules. The liver contains a great deal of fat uniformly distributed in large and small globules in cells. The details of cell disease are hard to make out because of post-mortem changes. The liver is distinctly lobulated, and in some places slight fibroblastic proliferation has occurred along the lines separating hepatic lobules. Glycogen is absent.

Rabbit 28—Soybean diet; lived two hundred and sixty-six days; killed when weight began to fall, to see the liver. Weight at start 2,800 Gm., at death 4,000 Gm. (fig. 1). Received alcohol 50 cc. for forty-seven days, 60 cc. for twelve days, 70 cc. for ten days, 75 cc. for twenty-four days and 100 cc. for one hundred and thirty-two days, a total of 18,770 cc., or 3,754 cc. of absolute alcohol. Autopsy report: The rabbit is well nourished, with abundant fat. There are no gross lesions. The liver weighs 137 Gm. Microscopic observations: There is moderate diffuse fatty infiltration in small vacuoles in over half the cells; very little glycogen is present. The cells are irregular in size, some slightly larger than normal, many much smaller. There is definite cell atrophy in spite of the weight of the liver, the liver cords showing as narrow bent cords; the sinusoids are dilated, prominent, but not filled with blood. The cytoplasm of cells is often muddy, lumpy, coagulated into round irregular masses, not hyaline but taking the eosin stain. (The type of atrophy found in this animal, in rabbit 3 and in others can be seen in figure 7, from rabbit 32).

Rabbit 30.—Soybean diet; lived 224 days, died of alcoholism. Weight at start 4,200 Gm., at death 3,100 Gm. (fig. 4). Received 50 cc. of alcohol for ten days, 60 cc. for twenty days, 70 cc. for twenty-four days, 80 cc. for fifty days and 100 cc. for forty-two days; then again 80 cc. for thirty days and 70 cc. for thirty days, a total of 16,180 cc., or 3,236 cc. of absolute alcohol. Autopsy report: All the organs except the liver are normal in appearance. The spleen is not enlarged. The liver weighs 138 Gm., is pale and fatty, has a granular surface and cuts with some resistance to the knife. The cut surface shows lobules irregular in size, some larger than normal and easily seen with the naked eye. Circular bands and streaks of glistening hyaline material can be seen running throughout. All the lobes are affected to the same degree, looking alike. Microscopic observations: Nothing unusual is found except in the liver. In this organ two outstanding features strike the eye immediately, i. e., marked fatty infiltration and marked perilobular and portal fibrosis. The fat is present in large droplets, more toward the center of the lobule, less at the periphery. Each lobule is outlined by compressed cells or by thick or thin bands of fibrous tissue. The fibrous tissue in many places connects

one portal area with another, although in many other places there is little fibrosis around some lobules. Every section taken, all together representing every lobe of the liver, shows the same picture. There are hyaline degenerated areas, some within the lobules and many at the peripheries, adjacent to proliferating fibrous tissue. There are other, more acute necrotic areas where there is simply disintegration of cells. Both types of necrosis are associated with fatty cells, although those in which a hyaline type of degeneration is taking place are likely to contain less fat. There is remarkable proliferation of bile ducts in most portal areas. These are of somewhat more adult type than seen in cirrhosis in man, but most of them are not functioning; i. e., they consist of simple clumps of cells, frequently without lumens. There is more fibrous tissue around many of the portal areas than seen in cirrhosis in dogs, for instance. This has surrounded degenerating cells and included small islands of liver tissue within the fibrous tissue. The effect seems to be a true effect of the treatment given (figs. 5 and 6).

Rabbit 32.—Soybean diet; lived ninety-three days, died of alcoholism. Weight at start 2,400 Gm., at death 1,200 Gm. Received 50 cc. of alcohol for twelve days, 60 cc. for eighteen days; 50 cc. for eighteen days; 40 cc. for seven days; 30 cc. for seven days; 25 cc. for twelve days, a total of 3,370 cc., or 676 cc. of absolute alcohol. Autopsy report: The animal is greatly emaciated, without body fat. All the organs are essentially normal except the liver. This is small, weighing 37 Gm. and is of about normal color, but with the lobules distinctly outlined on the capsular and cut surfaces. It is tough and cuts with some resistance. Microscopic observations: The liver is distinctly lobulated throughout. In some places this is due to rows of atrophic liver cells around the peripheries; in many others the lobules are separated by wide or narrow bands of connective tissue producing definite perilobular or portal cirrhosis (fig. 7A). There are scattered small clumps of cells containing fat vacuoles, but most of the liver columns are shrunken, atrophic. Atrophied cells, most of them without nuclei, are squeezed between adjacent lobules or are caught between strands of proliferating connective tissue; they are represented in many places by syncytial masses of hyaline material or small homogeneous remnants of cytoplasm. Proliferation of the epithelium of bile ducts is marked in many portal areas, slight in some, and not present in others. Many focal areas of a peculiar type of acute degeneration are present. In these the cytoplasm of the cells has disappeared, and in most of the cells, the nuclei. What remains is a thick reticulum, staining blue with phosphotungstic acid-hematoxylin, forming outlines of what were cells, now mostly rounded or polyhedral spaces (fig. 7B). Frozen sections stained with scarlet red reveal no fat in these areas. (Note made at time of description: "This cirrhosis is as 'good' as that pictured by Mallory in phosphorus cirrhosis, though the gross appearance is not nearly so striking [Mallory, F. B.: *Am. J. Path.* 9:557, 1933, plate 93, fig. 11, and plate 94, fig. 13]. The focal necroses are exactly like those due to sodium arsenate shown by Von Glahn and associates [Von Glahn, W. C.; Flinn, F. B., and Keim, W. F.: *ARCH. PATH.* 25:488, 1938, fig. 1A]").

As for the whole group of rabbits, 20 had fatty infiltration as the predominant finding, 16 had liver cell atrophy, 4 showed acute necroses and 7 had fibrosis as the outstanding characteristic, although, as has been said, some livers showed several, and a few had all, of these lesions at the same time. Only 4 livers were declared normal.

Some effects of the administration of alcohol and of sugar to the rabbits on normal diets and those on soybean diets were investigated

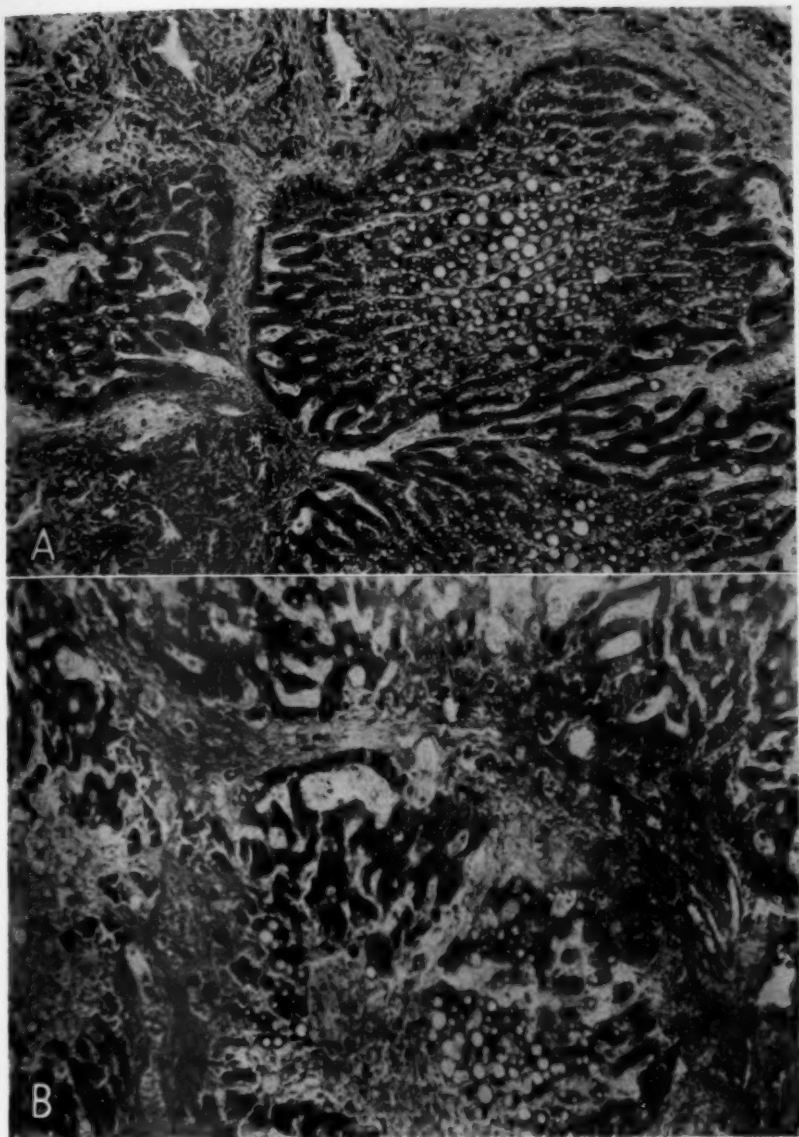


Fig. 5 (rabbit 30).—*A*, cirrhosis of a large liver, with considerable fat remaining; hematoxylin and eosin stain; $\times 89.5$. *B*, cirrhosis, fatty infiltration and necrosis of the same liver; hematoxylin and eosin stain; $\times 179$.

to the extent of determining the sugar tolerance under a variety of conditions. The tests were made after the animals had fasted from fourteen to sixteen hours, whatever treatment they had been on. The alcohol "addicts" were rabbits which had been on a daily dose of from 20 to 50 cc. of 20 per cent alcohol for a long enough period to acquire considerable tolerance. Several previous experiments had shown that when the rabbits were not fasting the blood sugar rose one hour after the administration of the usual dose of alcohol to a height of 140, 144 and 155 mg. per hundred cubic centimeters and remained high for longer than five hours, all of them having blood sugar above 135 at the end

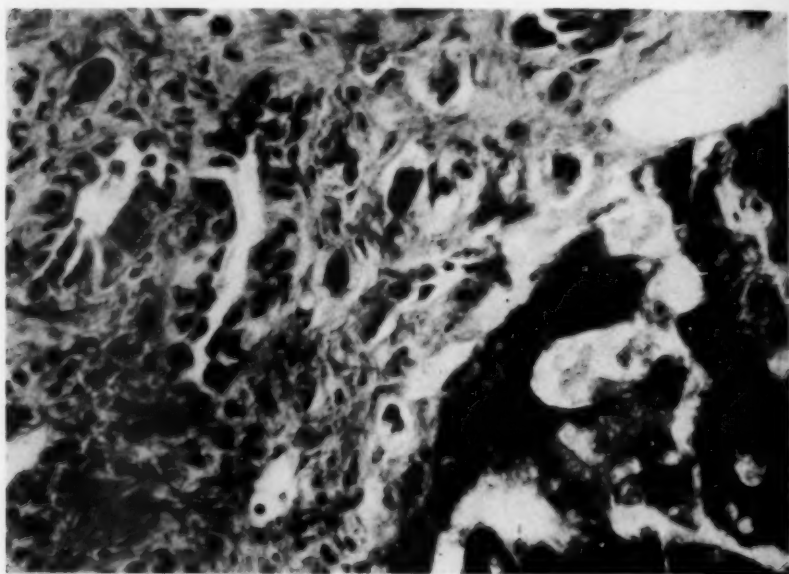


Fig. 6 (rabbit 30).—Edge of a lobule of the liver, showing degenerating liver cells and fibroblastic and bile duct proliferation; phosphotungstic acid-hematoxylin stain; $\times 447$.

of this period (normal 90 to 100)—this without having received dextrose. When the sugar tolerance curves were determined, dextrose was given in 10 per cent solution by stomach tube at the rate of 2.5 Gm. per kilogram, immediately after the fasting blood had been taken.

Figure 8 shows the blood sugar curves of rabbits on normal diets. Each graph represents the average of 2 rabbits except those for rabbits 3 and 4. It is assumed (it practically always is a fact) that at the end of the third hour the normal curve would have fallen below 100. The second curve, counting from below upward, at the end of the experiment was made twenty-four hours after these rabbits had had any alcohol. The addicts (third curve) which received 20 cc. of alcohol

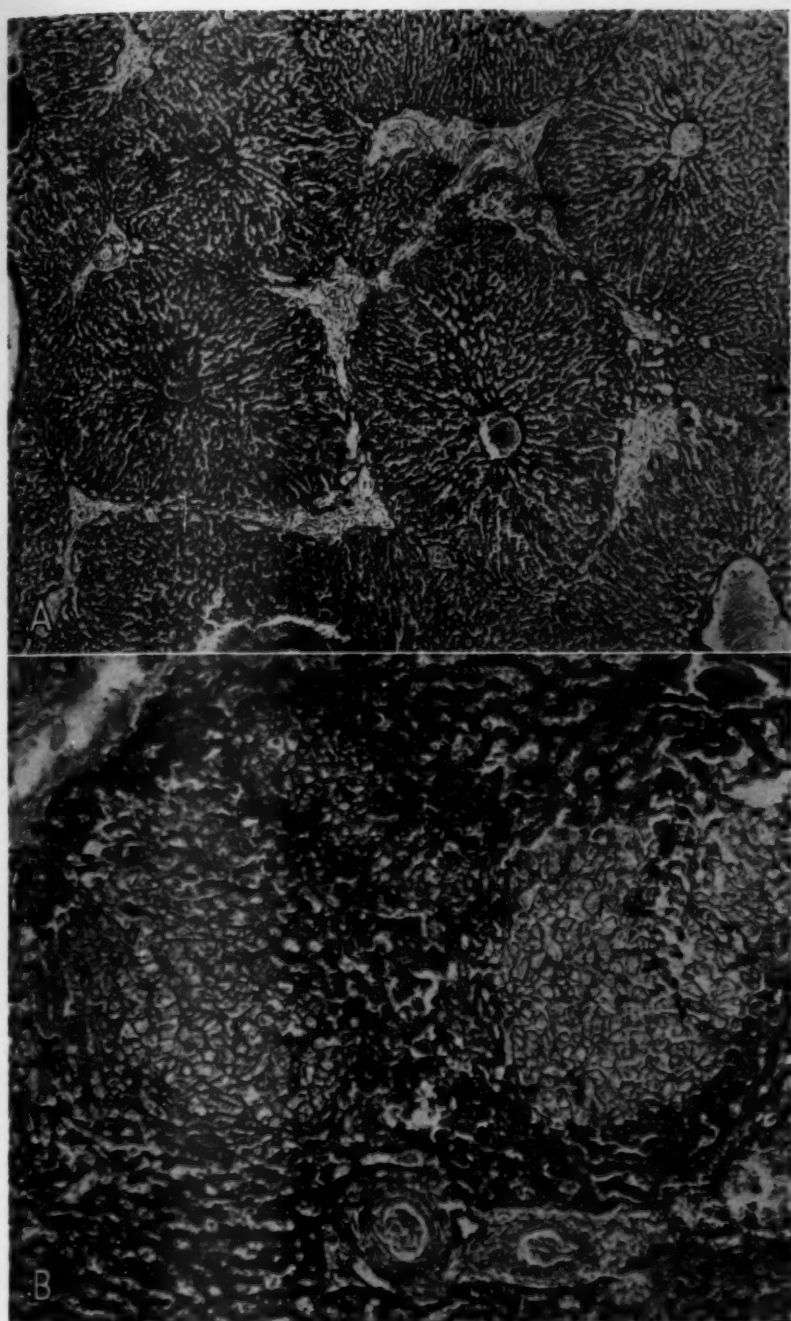


Fig. 7 (rabbit 32).—*A*, early cirrhosis in an atrophic liver weighing 37 Gm.; phosphotungstic acid-hematoxylin stain; $\times 68$. Perilobular fibrosis with atrophy of the liver cords is prominent. *B*, areas of necrosis in the liver shown in *A*; phosphotungstic acid-hematoxylin stain; $\times 178$.

solution one hour before dextrose was given have a tolerance similar to the normal rabbit (no. 3) which had never received alcohol except the 20 cc. solution one hour before the test, but when a dose of 50 cc. was given (rabbit 4), a progressively increasing amount of sugar accumulated in the blood and remained for an indefinite period, becoming at least as high as 240 mg. per hundred cubic centimeters.

Figure 9 shows the sugar tolerance of rabbits on the soybean diet, along with the same normal rabbits as in figure 8. However, the control rabbits on a soybean diet without alcohol at any time would form the "normal" controls for this group. In this instance rabbits which had been on daily doses of alcohol for several months (but had not received

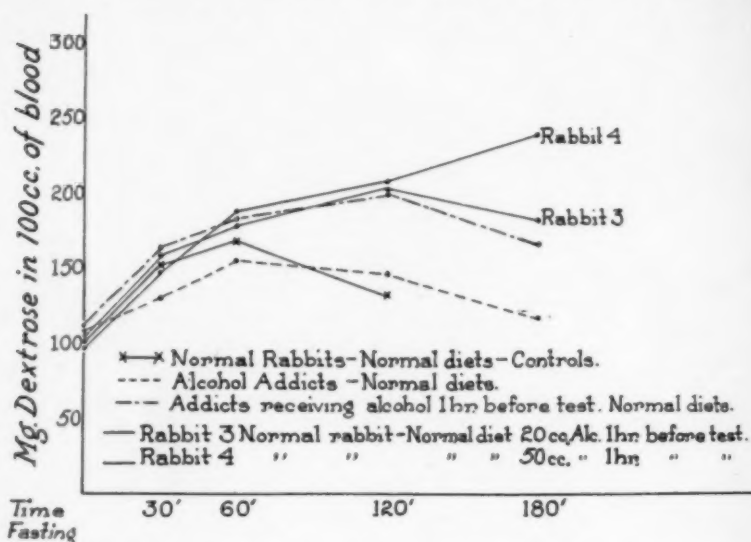


Fig. 8.—Blood sugar curves of rabbits on normal diets and alcohol. Additional details are given in the text.

alcohol for twenty-four hours) had about the same tolerance as those on the diet alone, and those which had been receiving 20 cc. of alcohol solution daily were not affected by the same dose given one hour before the test, but the tolerance was greatly reduced when over twice as much alcohol was given to these same animals one week later (top curve). During that week they had been receiving their daily doses of alcohol, so that the experimental conditions were the same. It appears that toleration to a given dose of alcohol both in this group and in the group on normal diets also carried with it some readjustment as to sugar tolerance. All the animals in both groups were eating their respective diets and were gaining or maintaining their weight, so that a starvation effect may be ruled out. The results indicate, as nearly as conclusions

can be drawn from this small number of tests, that repeated doses of alcohol at four or five hour intervals are necessary to maintain a continuous metabolic imbalance.

The first experiment, i. e., blood sugar curves taken when the animals had not been fasting, indicates that alcohol and a normal diet cause an abnormal accumulation of sugar in the blood, just as alcohol and dextrose do. It seems obvious that alcohol is responsible for this in the sugar tolerance group on normal diets and that the soybean diet augments the effect of alcohol in the second group. It might also be suggested that tolerance to alcohol seems very similar in nature to tolerance to a diet abnormally high in fat and protein.

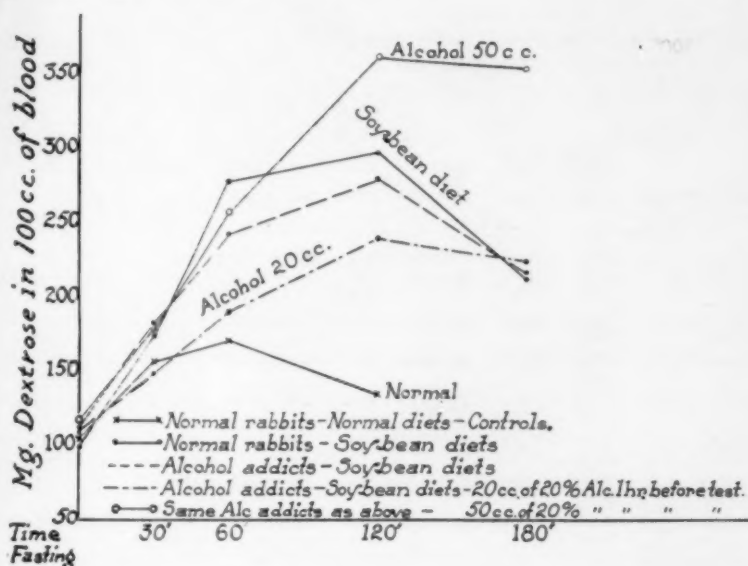


Fig. 9.—Blood sugar curves of rabbits on soybean diets and alcohol. Additional details are given in the text.

The question of the utilization of proteins, fats and carbohydrates was investigated by Carpenter¹ in acute experiments with alcohol, but the degree of inhibition of the metabolism of these substances in chronic severe alcoholism has not been fully determined. Himwich and co-workers⁷ showed that in man the blood sugar rises after ingestion of alcohol. Carpenter reported that alcohol caused a fall in carbohydrate metabolism and that when alcohol and dextrose were administered together the fall in the respiratory quotient was nearly the same as with alcohol alone. He wondered whether the disappearance of alcohol (from the expired air) was due to actual combustion of the alcohol or to transformation of the alcohol into some other substance.⁸

A year later LeLoir and Muñoz⁹ stated that 60 per cent of the alcohol in the liver was converted into acetic acid. From the chemical nature of alcohol and the manner in which it interferes with tissue respiration and the metabolism of genuine food substances its action may be compared to that of other toxic agents, notably lipoid solvents, and its effects to certain metabolic states produced by abnormal diets or by "spontaneous" diabetes mellitus. A table has been compiled so that these similarities may be more readily compared (table). Phosphorus is included in this group because of its probable inhibiting action on oxidizing enzymes, particularly those involved in carbohydrate metabolism, as well as because of its similar action on fat transference to the

*Similarities in the Action of Substances or the Influence of Conditions Which May Produce Cirrhosis of the Liver (Further Details in Text) **

	Blood Sugar	Blood Fat	Ketosis	Respiration Quotient	Liver Glycogen	Liver Change	
						Early	Chronic
Untreated diabetes mellitus	High	High	Present	0.71	Decreased	Fatty "hepatitis"	Fatty cirrhosis
Fasting.....	Low	High	Present	0.72	Decreased	Fatty	Atrophy
Excessive fat in diet	Low	High	Present	0.71 0.75	Decreased	Fatty	Fatty cirrhosis
Carbon tetrachloride	High Low	High	Present	0.70	Decreased	Fatty necrosis	Fatty atrophy, cirrhosis
Chloroform....	High Low	High	Present	Low	Decreased	Fatty necrosis	Fatty atrophy, cirrhosis
Methyl alcohol	High Low	High	Present	Low	Decreased	Fatty necrosis	Fatty cirrhosis
Alcohol.....	High Low	High	Present	0.667	Decreased	Fatty necrosis	Fatty atrophy, cirrhosis
Ether.....	High Low	High	Present	Low	Decreased	Fatty	?
Phosphorus....	High Low	High	Present	0.70	Decreased	Fatty necrosis	Fatty cirrhosis

* This table has been compiled from data accumulated in this laboratory and from the following sources: Beecher, H. K.: *The Physiology of Anesthesia*, New York, Oxford University Press, 1938. Browning, E.: *Toxicity of Industrial Organic Solvents*, New York, The Chemical Publishing Co., 1938. Joslin, E. P.: *Treatment of Diabetes Mellitus*, ed. 6, Philadelphia, Lea & Febiger, 1937. Sollmann, T.: *Manual of Pharmacology*, ed. 5, Philadelphia, W. B. Saunders Company, 1936.

liver, kidneys and muscles. It should be recognized that some of the results indicated may be transient or, in later stages of poisoning, reversed. For instance, the increased blood sugar after alcohol, ether, methyl alcohol, carbon tetrachloride, chloroform and phosphorus poisoning may fall to a level below normal and, at least in the last three types of poisoning, result in pronounced or even fatal hypoglycemia. By no means all patients with untreated diabetes have fatty livers, and in only a small percentage of those who have does the change go on to cirrhosis. Likewise, lipemia is present only in early acute alcohol

9. LeLoir, L. F., and Muñoz, J. M.: *Biochem. J.* **32**:299, 1938.

poisoning, and in this, as well as in most or all the other conditions cited, the lipid content of the blood drops to normal or below, certainly after the fat depots of the body have been depleted. The anatomic changes would vary also with the degree of intoxication and the length of time involved. One can hardly find better examples of the changed results produced by changing factors: the biochemical, physiologic and anatomic effects depending on the toxicity of the agent employed, the amount used and the length of time it is allowed to act. With these three changing factors to deal with, as well as a fourth, the incalculable tolerance of one animal over another, it can be seen that a given metabolic and anatomic condition may be true only for a given moment in the course of the experiment. Even the chronic irreversible end results in experiments with these substances are uncertain because of the extremely high mortality among the animals used to produce them. Toxic doses are necessary with all of them. A nontoxic dose of carbon tetrachloride will not produce cirrhosis of the liver any more than a nontoxic dose of alcohol will do so.

One can see from the table how, by adding alcoholism to preexisting diabetes or combining it with fasting or maintenance on a high fat diet, the physiologic effect would be enhanced. Also by adding alcoholism to phosphorus poisoning, as Bollman and Mann² did, or to carbon tetrachloride poisoning, as Lamson and Wing¹⁰ did, a greater summation of effects is obtained than by using one alone. Bollmann and Mann also showed that alcohol accelerates the accumulation of fat in the liver of a dog on a high fat diet, and Connor and Chaikoff¹¹ produced a fair degree of hepatic cirrhosis in dogs on a high protein, low carbohydrate diet after the livers had previously been made fatty by a high fat diet. In that experiment the fat diet was used mostly to shorten the time of development of fatty infiltration. A good many reports over a period of years have pointed out that depletion of glycogen from the liver by any means renders it more susceptible to these types of poisons, including alcohol. In view of the number of probable combinations of factors necessary to produce cirrhosis of the liver experimentally, the high mortality of animals receiving really toxic doses of alcohol and the previous disregard of these concomitant factors, as well as, knowing them, the difficulty in controlling them, it is not surprising that the number of animals getting cirrhosis in a given experiment is relatively small. The mortality in man himself performing the same experiment on himself is high, and the percentage which achieves actual cirrhosis is likewise small.

10. Lamson, P. D., and Wing, R.: *J. Pharmacol. & Exper. Therap.* **29**:191, 1926.

11. Connor, C. L., and Chaikoff, I. L.: *Proc. Soc. Exper. Biol. & Med.* **39**: 356, 1938.

NEUROPATHIC PULMONARY EDEMA

FURTHER OBSERVATIONS

SIDNEY FARBER, M.D.

BOSTON

Recent studies¹ demonstrated that acute pulmonary edema and death occur with regularity in rabbits and guinea pigs following bilateral cervical vagotomy. Evidence obtained from pertinent observations recorded in the literature and from these studies led to the suggestion that disturbances of the vasomotor control of the pulmonary vessels secondary to bilateral vagotomy in the rabbit or the guinea pig are responsible for important alterations in the dynamics of the pulmonary circulation and in the integrity of the walls of the pulmonary vessels. These alterations lead to severe congestion and acute edema of the lungs. The term "neuropathic" was employed to denote the genesis of this type of pulmonary edema.

The present studies were undertaken in order (1) to investigate the immediate effects of vagotomy on the lungs under different conditions, (2) to obtain further evidence concerning the genesis of neuropathic edema and (3) to measure the changes in blood volume caused by the loss of fluid from the lungs and upper respiratory tract.

METHODS—SERIES I-VI

Since a number of hours is required for the production of severe pulmonary edema in rabbits after bilateral cervical vagotomy, it was considered worth while to determine whether alterations of the pulmonary blood vessels in the lungs could be demonstrated immediately after vagotomy. For this purpose, a measured load on the cardiovascular system in the form of a rapid intravenous infusion of physiologic solution of sodium chloride was employed as a standard experimental procedure on intact rabbits and on rabbits subjected to bilateral cervical vagotomy, unilateral cervical vagotomy or the action of atropine sulfate.

Healthy rabbits varying in weight from 2,000 to 4,000 Gm. were employed in these studies. Local infiltration of the skin over the neck with procaine hydrochloride (1 per cent without epinephrine) was the anesthetic procedure used in all experiments unless otherwise stated. All necessary procedures (tracheotomy, insertion of a glass cannula into the jugular vein, and so on) were performed in such a manner that there was only minimal disturbance to the experimental

From the departments of pathology of the Harvard Medical School and of the Children's Hospital and the Infants' Hospital.

This work was supported by a grant from the William W. Wellington Memorial Research Fund of the Harvard Medical School.

1. Farber, S.: *J. Exper. Med.* **66**:397, 1937.

animal. When tracheotomy was performed, a tube of suitable bore was inserted into the trachea just below the thyroid level. When an animal was subjected to cervical vagotomy, the vagus nerves were freed with care to prevent stimulation of the central ends and were severed as low in the neck as possible. The aortic nerves were spared. A glass cannula of uniform size for insertion into either the right or the left external jugular vein was used in all experiments. The infusion fluid employed was warm physiologic solution of sodium chloride (0.85 per cent sodium chloride). The saline solution was administered from a 250 cc. infusion flask connected to the glass cannula by means of a rubber tube. The height of the infusion flask remained constant in all experiments. It was found impossible to adjust the rate of flow with sufficient exactness so that the same rate could be obtained in all experiments. Certain narrow limits in the rate of flow, however, were achieved. The rate of flow and the amount given intravenously were recorded in each case. The animal was placed on its back for the duration of the experiment, which was not begun until a satisfactory state of quiet behavior had continued for at least ten minutes. Animals were killed by injecting several cubic centimeters of 1 per cent procaine hydrochloride solution into the cisterna magna. Death occurred instantaneously.

SERIES I. RAPID INTRAVENOUS ADMINISTRATION OF PHYSIOLOGIC SOLUTION OF SODIUM CHLORIDE IN THE INTACT RABBIT

To secure data for the evaluation of the experiments to be described later, physiologic solution of sodium chloride was given intravenously to 10 rabbits (weighing 2,000 to 4,000 Gm.) at the rate of 30 to 40 cc. per minute to a total of 250 to 400 cc., over a period of time varying from seven to ten minutes. In five of these experiments a tracheal cannula was employed.

Results.—Soon after the onset of the infusion, in some rabbits after only 20 to 30 cc. of fluid had entered the vein, rapid shallow respirations were observed, and these continued throughout the period of the infusion. Two animals showed no other reaction to this rapid administration of fluid. In 8 rabbits respiratory distress occurred one to three times during the course of the infusion. This distress lasted but a few seconds in each case and was accompanied by a backflow of blood into the glass cannula. The backflow of blood (rise in venous pressure) occurred almost instantly after the onset of the respiratory distress and disappeared almost immediately after the resumption of quiet rapid shallow respiratory movements. During these periods of respiratory distress several strong convulsive movements of the entire body took place. At no time were rales heard on stethoscopic examination of the lungs. Occasionally urine was voided during the infusion. All animals appeared to be in good condition at the termination of the infusion. In 3 rabbits the rapid shallow respirations gradually became less marked during the next twenty minutes, at the end of which time these rabbits were killed. Four animals were killed from five to ten minutes after the fluid had been given.

The 7 animals killed from five to twenty minutes after the fluid was administered showed distention of the bladder, ascites (10 to 30 cc. of clear fluid), acute congestion of the liver and distention of the large veins in the thorax and abdomen. The lungs were pink to pale red and showed moderate diffuse congestion, most marked in the lower lobes. The trachea and bronchi contained no fluid. The cut surfaces of the lungs showed only congested vessels. There was no evidence of pulmonary edema. Three rabbits were permitted to survive twelve, eighteen and twenty-four hours after the conclusion of the experiment. These animals exhibited normal behavior after being returned to their cages. Postmortem examination

revealed no pathologic changes. No important differences, either on clinical observation or at postmortem examination, could be related to the use of the tracheotomy tube. An abstract of an average protocol follows:

Rabbit, 2.8 Kg. Tracheal cannula inserted. Animal on its back in position for experiment. Vagus nerves intact. Heart rate calculated from electrocardiogram.

Time, p. m.

- 3:25 Heart rate 240 per minute.
- 3:33 Intravenous administration of fluid begun.
- 3:36 100 cc. given. Heart rate 310 per minute. Rapid shallow respirations.
- 3:38 175 cc. given. Heart rate 260 per minute. Respiratory movements rapid and shallow.
- 3:40 240 cc. given. Heart rate 90 per minute. Intravenous infusion stopped. Respiratory movements rapid and shallow.
- 3:43 Heart rate 120 per minute.
- 3:49 Animal breathing quietly and less rapidly. It appears in good condition. Heart rate 150 per minute.
- 3:55 Animal in good condition. Killed by injection of procaine hydrochloride into cisterna magna. Postmortem examination reveals moderate congestion of lungs, most marked in the lower lobes. No fluid in trachea or bronchi.

SERIES II. RAPID INTRAVENOUS ADMINISTRATION OF PHYSIOLOGIC
SOLUTION OF SODIUM CHLORIDE IMMEDIATELY AFTER
BILATERAL CERVICAL VAGOTOMY

The details of technic are the same as those employed in series I, with the addition of bilateral cervical vagotomy, performed from two to ten minutes before the onset of the intravenous infusion. Twenty rabbits (weighing 2,000 to 4,000 Gm.) were used in this series. Tracheotomy was performed in 10 of these. In 2 experiments trypan blue was added to the saline solution in amounts sufficient to make a 1 per cent solution of the dye. Fluid was given to each rabbit until pulmonary edema occurred, the amounts varying from 135 to 350 cc. In general, the amounts required to produce pulmonary edema varied directly with the size of the rabbit. The majority received from 200 to 250 cc. at rates of 25 to 30 cc. per minute over a period of ten to fifteen minutes. The fluid was administered usually in smaller amounts at slower rates and over a slightly longer period of time than in series I, although attempts were made to duplicate the first series of experiments more exactly. Edema usually supervened before the planned amount of fluid could be given. The resistance to the entrance of fluid into the vein at the pressure employed was greater after the first few minutes of the infusion than in series I, which accounts for the slower rate of flow.

Results.—Immediately after bilateral cervical vagotomy the respiratory movements became slower and deeper. Crises similar to those described in series I were observed during the course of the infusion. They were characterized by a few convulsive movements of the entire body and a small series of rapid irregular respiratory movements. The onset was followed almost instantly by a backflow of blood into the cannula. These crises lasted usually for from three to five seconds. Each episode was succeeded by a resumption of slow deep respirations and quiet behavior. On some occasions a crisis was noted after 30 to 50 cc. of salt solution had been administered, and recurred three to five times during the infusion.

Rales could be detected on stethoscopic examination of the lungs after the infusion of 100 to 200 cc. in different experiments. In animals without tracheotomy the onset of severe edema could be recognized by the dripping of foamy fluid from the nose and mouth after a short period of increased respiratory rate and dyspnea. In tracheotomized rabbits foamy fluid appeared suddenly after a warning period of one to two minutes of increased respiratory rate and labored respiratory movements. The fluid was blown explosively, with each expiration, from the



Fig. 1.—Gross photograph showing congestion and severe edema in the lungs and opened trachea of a rabbit subjected to rapid intravenous infusion of physiologic solution of sodium chloride immediately after bilateral cervical vagotomy.

end of the tube and formed a small puddle of foamy fluid beside the tube. This explosive ejection of foamy fluid from the tracheotomy tube continued until the termination of the experiment. Rapid shallow respiratory movements comparable to those noted in series I, animals with intact vagus nerves, were never observed. In animals with tracheotomy tubes and with their esophagi tied, fluid dripped from mouth and nose during the period of progressive increase in the amount of pulmonary edema.

Twelve of the 20 rabbits in this series died within ten to twelve minutes after the onset of infusion, while the fluid was still entering the jugular vein. In general, the smaller rabbits died more quickly than the larger ones. Postmortem examinations performed at once revealed either no ascites or only slight moistening of the peritoneal surfaces, congestion of the liver and distention of the large veins, distention of the bladder, dilatation of the right ventricle and auricle, and heart chambers filled with fluid blood. The congestion of the liver and the distention of the large veins were not quite as marked as in series I. Pulmonary edema and congestion, both of severe degree, were present in all lobes of the lungs but were most marked in the lower lobes. The cut surfaces of consolidated gelatinous-appearing lungs dripped frothy fluid. The trachea and bronchi were filled with thin foamy fluid, which in some experiments was blood stained (fig. 1). The tracheotomy tube was examined in each case and was found free from obstruction. There were no important differences noted in the postmortem examination of those with and those without tracheotomy tubes.

Three rabbits died spontaneously five to fifteen minutes after the cessation of infusion. Five animals, 3 with tracheotomy tubes and 2 without, and all adults, were killed by injecting procaine hydrochloride into the cisterna magna fifteen minutes after the infusion had been completed. These animals were dying from obvious, severe pulmonary edema. The postmortem examinations showed essentially the same changes as those already recorded. In the 2 rabbits which received trypan blue in the saline solution the trachea and bronchi contained blue-stained foamy fluid. The parenchyma of the stained lung dripped foamy colored fluid.

The absence of ascites and the presence of marked pulmonary edema and congestion were the two most important differences between series I and series II.

An abstract of an average protocol follows:

Rabbit, 3 Kg. Tracheotomy. Heart rate calculated from electrocardiogram.

Time, p. m.

- 5:35 Heart rate 260 per minute. Animal lying quietly on its back.
- 5:41 Left cervical vagotomy. Heart rate 240 per minute.
- 5:43 Right cervical vagotomy. Heart rate 290 per minute. Slow deep respiratory movements.
- 5:45 Infusion into right external jugular vein begun.
- 5:49 100 cc. given. Heart rate 300 per minute.
- 5:52 190 cc. given. Heart rate 310 per minute. The respirations are now more labored and more rapid than at any time since vagotomy. A few crises with convulsive movements are noted. These are followed immediately by a transient rise in venous pressure.
- 5:45 230 cc. given. Heart rate 290 per minute. Rise in venous pressure is noted. Fluid is ejected in spurt from the tracheotomy tube with each expiration.
- 5:55 240 cc. given. The infusion is stopped. Heart rate 310 per minute. Foamy fluid continues to be forced through the tracheotomy tube.
- 5:57 The animal appears moribund. Severe pulmonary edema is present. Heart rate 250 per minute.
- 5:58 Respirations are now extremely labored; only occasional gasps are made.
- 5:59 Respirations have stopped. Heart rate 180 per minute.
- 6:02 Postmortem examination begun. Lungs deeply congested and edematous. Trachea and bronchi filled with foamy fluid.

SERIES III. RAPID INTRAVENOUS ADMINISTRATION OF PHYSIOLOGIC SOLUTION OF SODIUM CHLORIDE IN THE INTACT RABBIT, FOLLOWED BY BILATERAL CERVICAL VAGOTOMY AND THEN A SMALL INTRAVENOUS INFUSION

Five rabbits were prepared as in series I. The vagus nerves were intact. From 200 to 250 cc. of fluid was injected within eight to ten minutes. No evidence of pulmonary edema could be detected. Bilateral cervical vagotomy was then performed. Immediately thereafter, on infusion of an additional 50 to 100

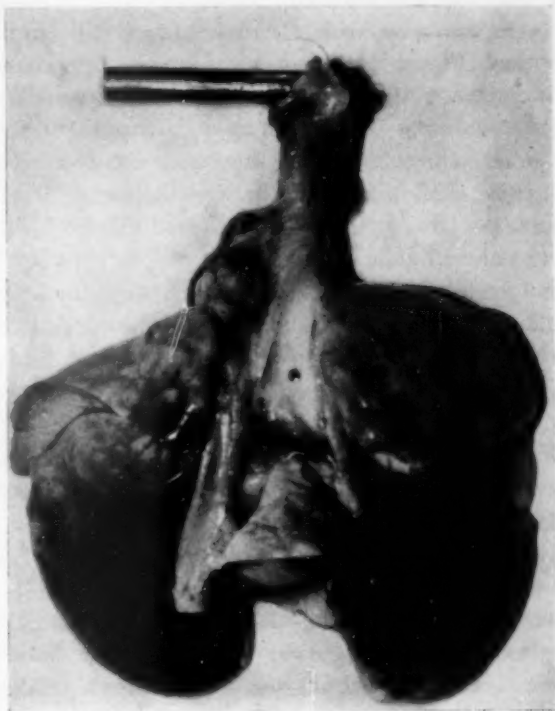


Fig. 2.—Gross photograph showing congestion and edema in the lungs and partially opened trachea of a rabbit subjected first to rapid intravenous infusion of saline solution and then to bilateral cervical vagotomy.

cc. of fluid during the next three to five minutes, acute pulmonary edema occurred. Postmortem examination revealed marked pulmonary edema and congestion in amounts similar to those observed in series II (fig. 2).

COMMENT ON SERIES I TO III

The purpose of the experiments in series I was to determine the amount of fluid which could be given intravenously to an intact rabbit within a short period of time without causing either death or important pulmonary changes. Fluid was given in amounts and at rates great

enough to produce a moderate degree of pulmonary congestion and occasional crises, characterized by a few convulsive movements, which were followed almost immediately by a transient rise in venous pressure. The effects on the cardiovascular system of intravenous infusion have been studied by a number of workers. Warthen² found that massive infusions were required in the dog before pulmonary edema could be produced. No pulmonary edema was produced in a medium-sized dog by Wiggers³ injecting 15 liters of saline solution intravenously within three hours (83 cc. per minute) unless pulmonary pressures were increased further by epinephrine. Altschule and Gilligan⁴ found that rapid intravenous infusion of physiologic solution of sodium chloride in man causes an increase in pulmonary blood volume, vasodilatation, a rise in venous pressure and an increase in cardiac output, but no pulmonary edema, in normal subjects. During the rapid intravenous infusion of salt solution, rapid shallow respirations were noted in the animals with intact vagus nerves but not in the vagotomized rabbits. This observation may be explained by the demonstration of Churchill and Cope⁵ that rapid shallow breathing associated with congestion of the lungs is caused by the stimulation of the vagal endings within the lungs. Similar conclusions were reached by Harrison and his co-workers,⁶ who observed that the rapid breathing found in various diseases of the thoracic organs is essentially of reflex origin. These reports recall the observation by Porter and Newburgh,⁷ in 1917, that cocainizing the vagus nerves of the dog changes the violent dyspnea of pneumonia into quiet normal breathing. Stimulation of the vagus nerves accounts also for the slowing of the heart observed during the intravenous infusion in the intact rabbit in the experiment cited.

The results of the experiments described are in general agreement with those reported by Kraus⁸ and Brunn⁹ on the basis of similar studies. Kraus concluded that the pulmonary edema produced by the intravenous administration of saline solution in the recently vagotomized rabbit or cat may be explained by the loss of the centripetal fibers of the pulmonary vasomotor nerves. He suggested that the resulting distur-

2. Warthen, H. J.: *Arch. Surg.* **30**:199, 1935.

3. Wiggers, C. J.: *Physiology in Health and Disease*, ed. 3, Philadelphia, Lea & Febiger, 1939, p. 695.

4. Altschule, M. D., and Gilligan, D. R.: *J. Clin. Investigation* **17**:401, 1938.

5. Churchill, E. D., and Cope, O.: *J. Exper. Med.* **49**:531, 1929.

6. Harrison, T. R.; Calhoun, J. A.; Cullen, G. E.; Wilkins, W. E., and Pilcher, C.: *J. Clin. Investigation* **11**:133, 1932.

7. Porter, W. T., and Newburgh, L. H.: *Am. J. Physiol.* **43**:455, 1917.

8. Kraus, F.: *Ztschr. f. exper. Path. u. Therap.* **14**:402, 1913.

9. Brunn, F.: *Wien. klin. Wchnschr.* **46**:1426, 1932.

bances in the regulation of blood flow lead indirectly to edema, but are essential in the genesis of this type of edema. Failure of the left ventricle was not demonstrated in his experiments. Brunn observed that morphine or posterior pituitary hastened the onset of the edema produced by vagotomy plus slow intravenous infusion of 1 per cent saline solution. If the kidneys were removed or denervated before the vagus nerves were cut and fluid given intravenously, Brunn was unable to produce pulmonary edema. Further investigation is required for the explanation of this observation.

It is probable that a number of cardiovascular and pulmonary mechanisms are altered or disturbed by bilateral cervical vagotomy in the rabbit. Some difficulty is encountered in the evaluation of the exact importance of these in the production of pulmonary edema under the experimental conditions employed in these studies. Some of these mechanisms will be discussed.

Rapid shallow respirations were not observed in vagotomized rabbits during the course of the infusion. The respiratory movements, on the contrary, became slow and deep immediately after vagotomy, a change attributable directly to denervation of the lungs (Anrep and Samaan¹⁰). The inability of the vagotomized rabbits to react by rapid respiratory movements to the increase in pulmonary blood flow probably favors the development of more intense congestion than occurs as a consequence of infusion in the intact rabbit. Of importance in the explanation of the increase in respiratory rate in vagotomized animals after the onset of pulmonary edema in the present experiments is the observation of Harrison that a diminution in vital capacity in vagotomized dogs is not followed by rapid breathing usually unless the diminution is of sufficient degree to produce marked lack of oxygen or an increase in the acidity of the blood. Anoxia caused by the severe edema was an important terminal factor in the present experiments and probably intensified the already existing pulmonary edema.

Cervical vagotomy deprives the animal of the function of the nerve endings in the walls of the venae cavae and the pulmonary veins, recently localized by Nonidez.¹¹ The nerve endings in these locations are responsible for the acceleration of the cardiac rate induced by a rise in the pressure of blood entering the right auricle (Bainbridge reflex). This acceleration is produced by stimulation of the afferent vagal endings in the walls of the great veins; it permits the adjustment of the heart's rate to the volume of blood poured into its chambers (venous inflow). The importance of this reflex in man is questioned, although it appears to be

10. Anrep, G. V., and Samaan, A.: *J. Physiol.* **77**:1, 1932.

11. Nonidez, J. F.: *Am. J. Anat.* **61**:203, 1937.

of importance in cats and dogs.¹² Sassa and Miyazaki¹³ were unable to elicit this reflex in rabbits even by the injection of rather large amounts of fluid with a considerable rise in the venous pressure. If this reflex mechanism is effective at all in rabbits, it is probably not of primary importance in the production of this type of pulmonary edema.

In recent experiments on dogs Schwiegk¹⁴ found that branches of the pulmonary artery possess nerve endings responsible for reflex pressosensitivity. This mechanism regulates the cardiac rate and arterial vasomotor tone; an increase in the pressure within the pulmonary arterial system causes reflex slowing of the heart and vasodilatation; lowering of the pressure within the pulmonary arteries is responsible for opposite cardiovascular reactions. These reactions cannot be elicited if the nerve fibers from the pulmonary arteries which connect with the vagus nerves are severed. Schwiegk expressed the belief that during periods of pulmonary congestion the reflex lowering of the general blood pressure caused by the rise in the pulmonary arterial pressure is accompanied by peripheral and splanchnic vasodilatation, which tends to prevent overloading of the pulmonary circulation. It may be suggested also that at times of temporary increase in the pressure in the pulmonary artery such a mechanism might exert a beneficial influence on the pulmonary circulation by preventing an increase in pressure in the bronchial artery. The exact importance of this reflex mechanism must await further work.

The effect on the heart itself following vagotomy may be mentioned. In the rabbit there is no important change in the rate of the heart after vagotomy. Previous studies¹ have shown that the heart plays no primary role in the production of pulmonary edema following bilateral vagotomy. Kraus⁸ observed no evidence of left ventricular failure in experiments similar to those reported here. Pulmonary edema occurred when the arterial pressure was at a normal level. The right ventricle is affected after the onset of pulmonary edema (dilatation). The experimental conditions employed in the present studies were designed to add a burden to the circulatory system. No evidence was obtained, however, that heart failure occurred before the onset of the pulmonary edema. It is implied from this discussion only that the heart does not play a primary role in the production of this form of pulmonary edema. It is evident that a secondary and probably terminal effect on the heart must obtain when the lungs are damaged by pulmonary edema at the time that a burden is put on the circulation by a rapid increase in blood volume.

12. Altschule, M. D.: *Medicine* **17**:75, 1938.

13. Sassa, K., and Miyazaki, H.: *J. Physiol.* **54**:203, 1920.

14. Schwiegk, H.: *Arch. f. d. ges. Physiol.* **236**:206, 1935.

There remains to be discussed the loss, following vagotomy, of the function of the vasomotor fibers of the vagus which control the pulmonary vessels. In the rabbit, it has been shown by von Euler,¹⁵ stimulation of the cervical vagus nerves causes constriction of the pulmonary vessels. Acetylcholine exerts a similar effect. Evidence obtained from a long series of ingenious experiments performed by de Burgh Daly and his co-workers¹⁶ suggested that pulmonary vasomotor nerves may maintain a more or less constant pulmonary arterial pressure at all rates of circulation and thus prevent overloading of the right side of the heart. Wearn¹⁷ demonstrated that the whole of the vascular bed is not open at one time except when the blood flow is very rapid. It has been suggested, therefore, that a second function of the pulmonary vasomotor nerves is the control of the distribution of blood within the lungs. The loss of these functions following vagotomy appears of the greatest importance in the production of the acute pulmonary edema observed in the present experiments. That an additional consequence of loss of vasomotor control of the pulmonary vessels is an increase in the permeability of the capillaries has been suggested by previous experiments. Whether such an increase in permeability precedes or, as appears more likely, accompanies the alterations of blood flow within the lungs after disturbance of the vasomotor mechanism cannot be stated, because of the difficulty in separating and studying individually the several consequences of loss of vasomotor control of the pulmonary vessels. These alterations secondary to loss of vasomotor control of the pulmonary vessels require a period of hours for the production of pulmonary edema in the vagotomized rabbit. In the present experiments, when the effects of a rapid increase in the blood volume (increase in capillary pressure) were superimposed on vagotomy, pulmonary edema could be produced within a few minutes. Conversely, when the consequences of vagotomy were added to the effects produced by a rapid increase in blood volume, which alone did not cause edema, acute pulmonary edema occurred without delay.

Acute pulmonary edema has been produced following simple bilateral cervical vagotomy by Weiser¹⁸ in the rat, and in experiments previously described,¹ in the rabbit and the guinea pig. Grady,¹⁹ at the suggestion of Moon, observed similar experimental results in the rabbit. In a recent study of the consequences of bilateral cervical vagotomy, carried out mainly on the rat, but also on the rabbit and the guinea pig, Lorber²⁰

15. von Euler, U. S.: *J. Physiol.* **74**:271, 1932.

16. de Burgh Daly, I.: *Physiol. Rev.* **13**:149, 1933

17. Wearn, J. T.; Ernstone, A. C., and Bromer, A. W.: *Am. J. Physiol.* **85**:410, 1928.

18. Weiser, J.: *Arch. f. d. ges. Physiol.* **231**:68, 1932.

19. Grady, cited by Moon,²⁴ p. 347.

20. Lorber, V.: *J. Exper. Med.* **70**:117, 1939.

concluded that obstructive asphyxia, secondary either to laryngeal paralysis or to collection of mucus in the tracheal cannula, plays a dominant role in the production of the pulmonary changes following vagotomy and that loss of vagal innervation of the lungs is of no more than secondary importance. A number of methods were utilized in previous studies to exclude laryngeal paralysis and obstructive asphyxia from the experimental conditions employed. In the present experiments, obstructive asphyxia and laryngeal paralysis were excluded without difficulty in the short time (less than ten minutes) required to produce severe pulmonary edema. Lorber did find that the effects of vagotomy are not negligible, since paralysis of the larynx caused by section of the recurrent laryngeal nerves can be tolerated for many days by rats which will die within three to three and one-half hours if the vagus nerves are severed in the neck. Weiser had found that rats die within three to six hours after bilateral cervical vagotomy.

Of considerable interest is the observation made by Lorber that intrathoracic vagotomy with sparing of the recurrent laryngeal nerve on one side and cervical vagotomy performed ten to fourteen days later on the other side permit almost indefinite survival in some, but not all, small animals subjected to these procedures. Evaluation of this observation must await a repetition of the experiment with both steps performed at the same time or within a short interval to eliminate the complicating factors incident to the ten to fourteen day delay between section of the first nerve and section of the second. Respiratory obstruction was found the cause of death in animals which failed to survive. Since the laryngeal nerve on one side was spared, it is difficult to understand the reason for the occurrence of respiratory obstruction sufficient to cause death in all the rats and in some of the guinea pigs and rabbits on which this two stage operation was performed. We have observed that guinea pigs and rabbits subjected to unilateral cervical vagotomy, either on the right or on the left, survived in good condition for months, without evidence of respiratory obstruction even when precautions were taken to prevent regeneration by removing a long segment of the nerve in the long term experiments.

The experiments in series II and III demonstrate an immediate and drastic effect of vagotomy on dilated pulmonary vessels. An analysis of the available evidence suggests that a number of processes initiated by bilateral vagotomy are responsible for the production of acute pulmonary edema when the blood volume is increased rapidly, but that alterations in the pulmonary vessels secondary to loss of vagal innervation are of primary importance. Whatever the exact mechanism, it is clear that a causal relationship exists between the vagotomy and the acute pulmonary edema under the conditions employed in these studies.

SERIES IV. RAPID INTRAVENOUS ADMINISTRATION OF PHYSIOLOGIC
SOLUTION OF SODIUM CHLORIDE IN INTACT
ATROPINIZED RABBITS

Seven rabbits were used in this series. Two rabbits, given 2 mg. per kilogram and 1.75 mg. per kilogram, respectively, of atropine sulfate in the marginal vein of the ear, died suddenly just after the infusion was started. Five rabbits were then prepared with intravenous injections of atropine sulfate in doses of 1 mg. per kilogram. From 250 to 375 cc. of salt solution was employed in these experiments. The fluid was injected at the rate of 25 to 40 cc. per minute over a period of ten to twelve minutes, within five to ten minutes after the atropine had been administered. The findings in this series duplicated those in series I. Rapid shallow respirations were the rule during the course of the infusion. No pulmonary edema was noted either clinically or at postmortem examination. All animals were killed by injecting procaine hydrochloride into the cisterna magna either at the termination of the infusion or within ten minutes thereafter. Small amounts of clear fluid were found in the peritoneal cavity. Generalized congestion was present.

SERIES V. RAPID INTRAVENOUS ADMINISTRATION OF PHYSIOLOGIC
SOLUTION OF SODIUM CHLORIDE IN THE ATROPINIZED RABBIT
FOLLOWED BY BILATERAL CERVICAL VAGOTOMY AND
A SMALL INTRAVENOUS INFUSION

To investigate the differences between the effects of atropine and vagotomy, 5 rabbits were treated as in series IV with atropine sulfate in doses of 1 mg. per kilogram. From 200 to 250 cc. of fluid was injected intravenously within eight to ten minutes. No evidence of pulmonary edema could be detected. The vagus nerves were then cut, and 50 to 75 cc. of fluid was injected intravenously immediately thereafter. During the course of the next two to three minutes, or by the time this amount of fluid had passed into the jugular vein, rales could be heard in the lungs, and the ejection of foamy fluid from the tracheotomy tube with expiration could be observed. Postmortem examination revealed marked pulmonary edema and congestion in amounts similar to those observed in series II (fig. 3).

COMMENT ON SERIES IV AND V

Atropinized rabbits given saline solution intravenously at rates and in amounts sufficient to produce pulmonary edema in recently vagotomized animals presented no more changes than were noted in animals with intact vagus nerves which received comparable infusions. Section of the vagus nerves in atropinized animals which had been subjected to rapid intravenous infusion of fluid was followed immediately by pulmonary edema if additional small amounts of saline solution were given. There is other evidence that the action of atropine sulfate does not duplicate the consequences of simple cervical vagotomy. A small series of experiments was carried out on both guinea pigs and rabbits. Large amounts of atropine sulfate (from 1 to 2 mg. per kilogram) were administered either intraperitoneally or intravenously to 4 guinea pigs and

4 rabbits at intervals of one to two hours or, in some cases, over a period of several days. Repeated injections were made, since the effect of atropine passes off more quickly in rabbits than in other animals. No more than four injections were made on any one day. The animals were permitted to remain in their cages, where they exhibited normal behavior. When they were put to death, no evidence of pulmonary edema was found.



Fig. 3.—Gross photograph showing congestion and severe edema in the lungs and partially opened trachea of an atropinized rabbit subjected first to rapid intravenous infusion of saline solution and then to bilateral cervical vagotomy.

It was formerly believed that atropine did not paralyze vasomotor fibers. It is clear from the experiments of Luckhardt and Carlson²¹ that atropine in pharmacologic doses causes vasomotor paralysis in the frog and in the turtle. These workers showed also that the pulmonary vasomotor fibers were paralyzed more quickly by atropine than were the bronchomotor fibers. Von Euler¹⁵ found in the rabbit that the constrict-

21. Luckhardt, A. B., and Carlson, A. J.: *Am. J. Physiol.* **56**:72, 1921.

tion of the pulmonary vessels caused by stimulation of the vagosympathetic nerves could be prevented by atropine. If it is assumed that atropine abolishes the actions of the pulmonary vasoconstrictor fibers in the intact animal, two possibilities suggest themselves: either the effects of vagotomy on the lungs of the rabbit are not brought about through loss of vasomotor control of the pulmonary vessels or the effects of atropine on the vasomotor fibers do not equal in intensity the complete loss of vasomotor control secondary to vagotomy. Evidence in favor of the latter possibility is found in the experimental production of pulmonary edema in the guinea pig when only the pulmonary fibers of the vagus are destroyed. In the present studies the effects of atropine did not duplicate those of bilateral vagotomy in respect to the production of pulmonary edema. These observations suggest that atropine does not abolish completely the vagal vasomotor mechanism in the lungs of the intact rabbit.

SERIES VI. RAPID INTRAVENOUS ADMINISTRATION OF FLUID TO RABBITS SUBJECTED TO UNILATERAL VAGOTOMY

Five rabbits in which tracheotomy had been performed were subjected to unilateral cervical vagotomy, on the right side in 3 and on the left in 2. Physiologic solution of sodium chloride was administered intravenously in amounts and at rates similar to those used in series I. All animals were in good condition at the termination of the infusion and were killed five to ten minutes later. Uniform results were not obtained in this small series. In 1 rabbit only mild congestion, most marked in the lower lobes of the lungs, was noted. This was not greater than the amount noted in series I. In 3 rabbits there were moderate edema and congestion in both lower lobes. No fluid was found in the trachea or bronchi. In 1 animal subjected to right vagotomy there was congestion of both lower lobes, with slight edema of the right lower lobe. There was a small amount of fluid in the trachea.

COMMENT ON SERIES VI

The results of the experiments in series VI are not conclusive but suggest that further observations might be worth while. Unilateral vagotomy alone causes neither death nor pulmonary edema. It has been mentioned that the rabbit and the guinea pig survive indefinitely after a portion of the vagus nerve in the cervical region on either side is removed. It is known that there is cross innervation of the lungs by the vagus nerves, although considerable variation exists in the amount of cross innervation. Such variation may explain the congestion and edema in both lower lobes in 3 rabbits in this series although it might be expected that the effects would be greater in the homolateral lung, as was the case in one experiment. A similar explanation for the finding of congestion only in one experiment suggests that a sufficient degree of protection was given to the contralateral lung by virtue of cross innerva-

tion. The position of the animal, on its back, accounts probably for the greater degree of congestion in the lower lobes of the lungs in all the experiments described, including those in this series.

SERIES VII. ALTERATIONS IN PLASMA VOLUME AND BLOOD
VOLUME SECONDARY TO PULMONARY EDEMA PRODUCED
BY BILATERAL CERVICAL VAGOTOMY

In earlier experiments, described in 1937, it was noted that the rabbits which died of pulmonary edema usually eight to twenty-four hours after bilateral cervical vagotomy lost a considerable amount of fluid through the lungs and upper respiratory passages before death and that at autopsy large amounts of fluid were present in the lungs, trachea and bronchi. It was considered worth while, therefore, to study the alterations in the blood.

Methods.—Experimental procedures similar to those described in a previous communication were used. Healthy rabbits were selected. At different times from three to seven days before the vagus nerves were cut low in the neck under local cutaneous anesthesia, induced with procaine hydrochloride, initial determinations were made of the plasma volume and the blood volume of the particular animal. After three to seven days a second series of observations was made. The vagus nerves were then cut as low in the neck as possible, the skin was closed with clips, and the animals were returned to their cages. At periods from five and a half to twenty-five hours after section of the vagus nerves, determinations of the plasma volume and the total blood volume were made once more. In 2 of the animals it was possible to make more than one determination before death. The data were collected in collaboration with J. G. Gibson 2d and T. H. Ingalls.

At intervals after section of the vagus nerves, the animals exhibited crises, which terminated with the expulsion of frothy and sometimes hemorrhagic fluid. In every case the crisis was followed by a period of quiet behavior and less labored respirations. A postmortem examination was made in each case. The most important changes were severe pulmonary edema, congestion and sometimes hemorrhage into the lungs.

Blood volumes were determined by the method developed by Gregersen, Gibson and Stead²² and modified by Gibson and Evans.²³ The determination of plasma volume is based on the dilution in the blood stream of an intravenously injected, accurately measured amount of dye of known color strength. The dye concentrations of four venous blood serum samples taken at five to ten minute intervals starting ten minutes after the injection of the dye were recorded in each experiment. The actual determinations were made by Dr. Gibson. The total blood volume is calculated from the plasma volume and the hematocrit reading. The red cell volume is taken as the difference between the volume of plasma and that of blood. Before the present experiments were undertaken, blood volume determinations were made on 10 normal rabbits for the adaptation of the technic to rabbits.

22. Gregersen, M. I.; Gibson, J. G., Jr., and Stead, E. A.: *Am. J. Physiol.* **113**: 54, 1935.

23. Gibson, J. G., Jr., and Evans, W. A.: *J. Clin. Investigation* **16**:317, 1937.

Results.—A summary of the findings is given in the table. In every experiment except one the plasma volume was reduced in percentages varying from 3.4 to 30.7. The average reduction for the group was 15 per cent. In one experiment (rabbit 5) the plasma volume was slightly increased from 93 to 95 cc. (2.2 per cent) five and one-half hours after the vagus nerves had been cut and two and one-half hours before death occurred. In this animal, however, the blood volume was reduced from 171 to 139 cc. The explanation for this was found in the clinical behavior of the animal. On several occasions following bilateral vagotomy the animal had short severe attacks of hemorrhage, which appeared to come from the trachea. At autopsy the trachea contained bloody frothy fluid, and on microscopic examination, in addition to the severe pulmonary edema, red cells in large numbers were present in the bronchioles and alveolar spaces. In another experiment (rabbit 3) it is of interest to note that the plasma volume

Summary of Results in Series VII

Rab- bit*	Observations Made		Survival Time After Vag- otomy, Hr.	Weight, Kg.	Plasma Volume		Hema- tocrit Read- ing	Red Blood Cell Volume		Total Blood Volume		
	Before Vag- otomy, Days	After Vag- otomy, Hr.			Cc.	Percent- age of Decrease		Cc.	Percent- age of Decrease	Cc.	Percent- age of Decrease	
1	A	7	3.41	165	33.7	84	249
	B	..	5½	20	3.41	136	17.6	31.9	64	23.8	200	19.7
2	A	3	3.75	148	38.3	92	240
	B	..	6	7	3.86	143	3.4	33.4	71	25.2	214	10.8
3	A†	6	3.00	132	34.8	70	202
	B	..	8	..	2.64	99	24.7	37.3	58	17.1	157	22.2
	C	..	25	42	2.64	108	17.9	30.4	47	32.8	155	23.2
4	A	7	2.42	101	33.5	41	152
	B	..	7	8	2.22	70	30.7	33.1	34	17.0	104	31.5
5	A	6	2.61	93	45.7	78	171
	B	..	5½	8	2.58	95	2.2‡	31.5	44	43.7	139	18.7

* Different observations on the same rabbit are indicated as A, B and C.

† The weight was estimated from the average figures for weight.

‡ This was an increase instead of a decrease.

taken eight hours after vagotomy was only 9 cc. more than that taken twenty-five hours after vagotomy. The blood volume, however, was considerably reduced; hemorrhage from the trachea occurred several times between the two readings. In every case the hematocrit reading was reduced. The red blood cell volume was likewise reduced (average reduction, 26.9 per cent), and the blood volume was consistently lowered (average reduction, 20.7 per cent).

It should be emphasized that none of these observations gives the true values on the blood which obtained at the time of death. The difficulty in predicting the time of death with accuracy, and the requirements which govern the collection of blood samples for the determination of plasma volume, made it impossible to obtain readings immediately before death. The pulmonary edema produced in the rabbit under these experimental conditions develops gradually for several hours and then increases rapidly. Terminal asphyxia caused by the pulmonary edema itself intensifies the pulmonary changes. It is therefore reasonable to assume that the plasma volume, and perhaps also the blood volume, which existed just before death, was considerably lower than that determined in these experiments.

COMMENT ON SERIES VII

A comparison of these findings with those characteristic of another type of pulmonary edema may be of interest. In a recent monograph on the shock syndrome, Moon²⁴ called attention to the occurrence of pulmonary edema in both man and experimental animals when death was delayed after the onset of shock. Emphasis was placed on the atony of the capillaries in this syndrome. Capillaries and venules dilate, and stasis develops. This withdraws a large volume of blood from circulation. There then occurs escape of fluid through the permeable walls of the capillaries and venules, which lowers the total blood volume, causes hemoconcentration and produces edema. Certain similarities are evident between the blood volume conditions described by Moon as characteristic of the shock syndrome and those in neuropathic pulmonary edema. In the experiments reported here the volume of the blood plasma and the volume of the total blood were lowered. The red cell volume, however, was decreased instead of being increased (hemoconcentration) as in Moon's observations. It should be mentioned that the red blood cell level is not constant in rabbits subjected to this experimental procedure. In another series of rabbits, not referred to in the foregoing record, both increase and decrease in the red blood cell count were observed, although increase occurred more frequently than decrease. The explanation for this variation lies in the fact that not all rabbits react in the same way during the development of neuropathic pulmonary edema. In some animals hemorrhage into and from the lungs is an important part of the picture; in others pulmonary hemorrhage is minimal or not apparent.

Although no further discussion is pertinent at this time, it may be stated that no similarity in the genesis of pulmonary edema occurring in shock and neuropathic pulmonary edema is implied by this discussion. Since the end result (pulmonary edema) and the immediate mechanism (increased permeability of vessels to blood plasma) are very much the same in the two forms of pulmonary edema, it is not surprising that the volume conditions in the blood stream may be similar.

SUMMARY

Acute pulmonary edema is produced in recently vagotomized rabbits within a few minutes after intravenous infusion of physiologic solution of sodium chloride in amounts and at rates which cause only moderate pulmonary congestion in intact rabbits and in atropinized rabbits with intact vagus nerves. When intact or atropinized rabbits in which moderate pulmonary congestion has been produced by rapid intravenous infusion of saline solution are subjected to bilateral cervical vagotomy

24. Moon, V.: *Shock and Related Capillary Phenomena*, New York, Oxford University Press, 1938.

followed by infusion of small additional amounts of the solution, acute pulmonary edema is produced without delay. Rapid intravenous infusion of saline solution into rabbits subjected to unilateral vagotomy causes either moderate congestion or congestion and slight edema. Tracheotomy has no demonstrable effect on these results. The action of atropine sulfate does not duplicate the consequences of bilateral cervical vagotomy in respect to the production of acute pulmonary edema. The plasma volume, red blood cell volume, blood volume and hematocrit reading become considerably reduced during the development of acute pulmonary edema caused by bilateral cervical vagotomy. The erythrocyte level may be above or below normal, depending on whether pulmonary hemorrhage occurs as a complication of the severe edema and congestion. The various factors concerned in the genesis of the acute pulmonary edema produced under the experimental conditions employed in these studies are considered and their relative importance discussed.

MENINGIOMA

NATHAN CHANDLER FOOT, M.D.

NEW YORK

After the publication of the excellent monograph by Cushing and Eisenhardt¹ on meningioma any immediate discussion of that subject might appear to be an act of supererogation. The uncertain "maternity" of these tumors, however, stimulates one to attempt to discover the originating cells and, other methods having only partially succeeded in accomplishing this, to endeavor to find some other line of approach to the subject.

A perusal of the classification of these tumors in the monograph just mentioned will show that they are composed chiefly of large cells arranged in bundles, masses or whorls that occupy the meshes of a scanty reticular stroma. Cushing and Eisenhardt collected 306 for the purpose of classification, and 121 of these showed this arrangement; 78 possessed whorls and showed a more marked tendency to form a reticular and collagenous stroma; 53 were largely composed of fibrous tissue in which the large pale cells became inconspicuous. The remaining 54 comprised angioblastic, malignant, chondroplastic, osteoplastic or lipoplastic forms that were too few to matter in connection with the point to be brought out.

This classification, then, infers the histologic nature of meningioma and arranges the tumors in a sort of gamut, at one end of which is the most usual epithelioid type and at the other those forms that express ascendancy on the part of the stroma. Thus one should immediately grasp the idea that these are in a manner compound tumors, a product of the proliferation of two sorts of tissue, the accent being now on one, now on the other, but usually on the solid complexes of cells. This is quite reasonable if one reflects that these neoplasms originate in the arachnoid villi, which are themselves composed of two groups of cells: the cap cells and the supporting stroma cells. This was first clearly recognized by M. B. Schmidt,² in 1902, but his ideas were rejected by Ribbert³ as "fantastic" and were lost to view until Cushing,⁴ in 1911.

From the Department of Surgical Pathology, Cornell University Medical College, and New York Hospital.

1. Cushing, H., and Eisenhardt, L.: *Meningiomas*, Springfield, Ill., Charles C. Thomas, Publisher, 1938.

2. Schmidt, M. B.: *Virchows Arch. f. path. Anat.* **170**:429, 1902.

3. Ribbert, M. W. H.: *Virchows Arch. f. path. Anat.* **200**:141, 1910.

4. Cushing, H.: *Brain* **45**:282, 1922.

in his Cavendish Lecture, described a meningioma that was accompanied by simple hypertrophy of the neighboring arachnoid villi and, drawing a comparison between the histologic pictures of the two, came to the conclusion that the tumor originated in similar villi.

Here one meets with immediate difficulties, as the origin of the arachnoid villi is still a matter of rather acrid dispute. While the majority agree that the tumor arises in the villi, the embryologic source of the cells that compose these is still a question. First considered as "endothelial," they were next termed "mesothelial," some authors still maintaining that they are just this. Mallory⁵ demonstrated "fibroglial fibrils" in meningocytes in 1920 and therefore called the tumors "arachnoid fibroblastomas." A little later Oberling⁶ proposed a novel concept, advancing the theory that the pia-arachnoid is really one syncytial membrane and not two separate entities. Among the cells composing this syncytium he distinguished larger cells (which he ultimately named "meningoblasts") and smaller cells, more like mesenchymal elements. He believed that the former migrated from the neural crest to the meninges, basing his assumption on a morphologic study of a 14 mm. pig and a 60 mm. human embryo. His ideas were enthusiastically received by Roussy, Masson and others but were as enthusiastically combated by Franceschini⁷ and Penfield,⁸ who took the mesodermal line of argument, Franceschini postulating a sort of polyblast as the parent cell, while Penfield espoused Mallory's fibroblastic theory.

Naturally, this dispute interested and ultimately involved the embryologists and anatomists. Weed⁹ experimented with embryos and concluded that the meninges were wholly a mesenchymatous product, but he was not too firm in ruling out from possible participation cells of the neural crest. Harvey and Burr¹⁰ transplanted the nervous system of *Amblystoma punctatum* into the flank of a larva of that species, including the neural crests in one case and excluding them in the other. In the latter instance no inner layer of large cells developed, the meninges failing to appear as such; in the former the reverse was true, and the meninges developed. Flexner¹¹ repeated their experiments in 1929 and obtained no confirmation of their results. Harvey, Burr and Van

5. Mallory, F. B.: *J. M. Research* **41**:349, 1920.

6. Oberling, C.: *Bull. Assoc. franç. p. l'étude du cancer* **11**:365, 1922.

7. Franceschini, P.: *Riv. di pat. nerv.* **39**:1, 1932.

8. Penfield, W.: *Tumors of the Sheaths of the Nervous System*, in *Cytology and Cellular Pathology of the Nervous System*, New York, Paul B. Hoeber, Inc., vol. 3, p. 953.

9. Weed, L. H.: *Contrib. Embryol.* **14**:225, 1917.

10. Harvey, S. C., and Burr, H. S.: *Arch. Neurol. & Psychiat.* **15**:545, 1926.

11. Flexner, L. B.: *Contrib. Embryol.* **20**:31, 1929.

Campenhout¹² thereupon repeated the experiments with variations. They transplanted the nervous systems of chick embryos into the allantoic sacs of others, with and without accompanying neural crests. Meninges developed only when these were present. They stained the neural crests of larvae of *Amblystoma* with Nile blue and transplanted them into the flanks of other larvae for further development. As this occurred, the large cells showed Nile blue granules; the granules were absent in the smaller mesenchymal cells. Next they transplanted the neural crests of larvae of *Rana palustris* into the flanks of larvae of *Amblystoma* after meticulously removing all *Rana* mesenchyma from the transplants. Only a few of their transfers developed, most of them being resorbed, but in those that did grow, in the scattered meningeal primordia, they found *Rana* cells and not the larger mesenchymal cells of *Amblystoma*, the host. Aware of these experiments, Raven¹³ removed small blocks of neural crest from the larvae of Triton and replaced them with exactly similar implants from the larvae of Axolotl. The implants healed promptly and continued to develop. By tracing the migrating cells from the implant, identified by means of their diameters (which differed materially from those of the host), he came to the conclusion that cells of the neural crest definitely participated in the formation of the meninges, thus confirming the work just cited.

Kredel¹⁴ approached the subject from the standpoint of tissue culture, coming to the astonishing conclusion that the meningocytes were macrophages. Buckley and Eisenhardt,¹⁵ using similar material and methods, found that meningioma was very refractory to cultivation and that it contained many macrophages in its stroma. These migrated and proliferated in tissue culture and misled Kredel to his strange conclusion. The only meningioma of the several that they attempted to cultivate which grew did so luxuriantly and was seen to be composed of spheroid cells with several bulb-tipped processes that anastomosed with one another and were unique in their appearance.

Stone¹⁶ had already experimented with the neural crest in 1921 and 1929 and found that a larva of *Rana palustris* deprived of its neural crests showed defective development of its gill arches and other hitherto presumably mesodermal derivatives. The neural crest cells were, therefore, multipotential and could produce cartilage and connective tissue. For this reason he spoke of the neural crest tissue as "mesectoderm."

12. Harvey, S. C.; Burr, H. S., and Van Campenhout, E.: *Arch. Neurol. & Psychiat.* **29**:683, 1933.

13. Raven, C. P.: *Arch. f. Entwicklungsmechn. d. Organ.* **134**:122, 1936.

14. Kredel, F. E.: *Am. J. Path.* **4**:337, 1928.

15. Buckley, R. C., and Eisenhardt, L.: *Am. J. Path.* **5**:659, 1929.

16. Stone, L. S.: *Anat. Rec.* **21**:85, 1921; *Arch. f. Entwicklungsmechn. d. Organ.* **118**:40, 1929.

Working along purely morphologic lines, Leary and Edwards¹⁷ attacked the problem in a very simple manner by making spreads of pleura, pericardium and peritoneum and comparing these with similar preparations of dura mater and pia-arachnoid. The procedure consisted of spreading out the unsectioned membranes on glass slides and treating them like microscopic sections, staining them and mounting them under cover slips. They were aware of Weed's ideas that the pia-arachnoid was a mesothelium; if he were correct, they argued, that membrane should not differ morphologically from the acceptedly mesothelial membranes just mentioned. They found a striking morphologic difference in the appearance of pia-arachnoid when compared with these; that they found the dura to be entirely different was, of course, to be expected.

This will give the reader an idea of the prevailing theories as to the origin of the arachnoid cap cells. Let us attempt to evaluate them.

Mallory's views were founded entirely on his examination of adult arachnoid villi, which revealed "fibroglia fibrils" in their cap cells together with collagen fibers in their stroma. To identify a cell by means of intracellular fibrils is hazardous; there is a good chance of error when drawing too sweeping conclusions. The collagen in the stroma is readily explained on the basis of a compound structure with a stroma and pseudoparenchyma. It does not prove that the latter is derived from the former. Both Mallory and Penfield dismissed the fact that the cells they called fibroblasts were acting in a manner quite foreign to fibroblasts elsewhere in the body. The masses, sheets and whorls are unlike anything formed by ordinary fibroblasts, so that one would have to postulate a subgroup of fibroblasts peculiar to the membranes of the nervous system before the theory would hold. This it is difficult to do. Another point speaking against this theory is the fact that meningocytes stain differently from fibroblasts and mesothelial cells in Masson preparations, taking on a deep pink color and an epithelioid solidity that is absent in those cells, which usually tend to be unsubstantial and greenish gray in sections treated with Masson's stain.

Oberling's theories were combated on the ground of lack of experimental evidence. Harvey, Burr and Van Campenhout have produced solid experimental confirmation of Oberling's views, bringing their data up into the warm-blooded group by employing chick embryos as well as amphibia. Thus one cannot say that their ideas are based on experiments with the latter alone and therefore do not apply to warm-blooded animals. As has been said, Weed was not categorically opposed to the participation of neural crest cells in the formation of the meninges. Flexner's failure to confirm the observations of Harvey and Burr

17. Leary, T., and Edwards, E. A.: *Arch. Neurol. & Psychiat.* 29:691, 1933.

remains unexplained. Raven's experiments confirmed them fully, and he expressed himself as convinced that they were correct. Stone's much neglected discovery of the multipotentiality of the neural crest cells opens new vistas and makes the whole controversy seem rather futile. One might conclude, then, that Oberling's ideas have been largely substantiated. Certainly the weight of experimental evidence tips the scale strongly in his favor.

There is another line of reasoning that might be applied in evaluating these theories: the place of the meningioma in the scale of other tumors that are morphologically similar. If one reflects on those neoplasms that tend to show a whorled structure, one immediately thinks of those that develop in the neural coverings. Beginning at the nerve terminals, one finds the melanoma characterized by whorled masses (best seen when it occurs in the scalp), which Masson called *lames foliacées*. Proceeding along the nerve trunks, one finds similar structures in the form of Verocay bodies of the neurilemoma and often a whorled radiating arrangement in the cells of the neurogenic sarcoma and the neurofibroma. Entering the spinal canal and cranium, one encounters the meningioma, which is characteristically whorled, and one may find similar arrangements in some forms of the glioma. Furthermore, all these tumors may occur more or less simultaneously in von Recklinghausen's disease, which is admittedly more readily attributed to nerve than to connective tissue disturbances. This has already been pointed out by Oberling and by Cushing. In such cases of the disease glioma or meningioma may constitute the terminal fatal lesion.

If one approaches the subject from the point of view of the "natural history" of these tumors, does it not seem more than mere coincidence that this morphologic feature should be common to this group? There are very few instances of any similar architecture occurring in tumors outside of it; some forms of the "pleural mesothelioma" show whorled architecture, and the "synovioma" described by Smith¹⁸ is somewhat similar and arises from mesothelium. This attracts attention to the mesothelial theory of meningeal origin, but there seems to be little doubt that the mesenchyme plays some part in meningeal histogenesis, so that too much weight should not be attached to this point.

Should these exceptions influence one too strongly, there are other points in common between tumors of the nervous system and the meningioma: They all tend to show some examples of pigmentation so far as Schwann cell or meningocytic participation is concerned. The production of melanin is chiefly a function of derivatives of the ectoderm, the main sources being the basal epithelium of the skin and the melanoblasts along the nerve sheaths. Certain brain cells have this potentiality.

18. Smith, L. W.: Am. J. Path. 3:355, 1927.

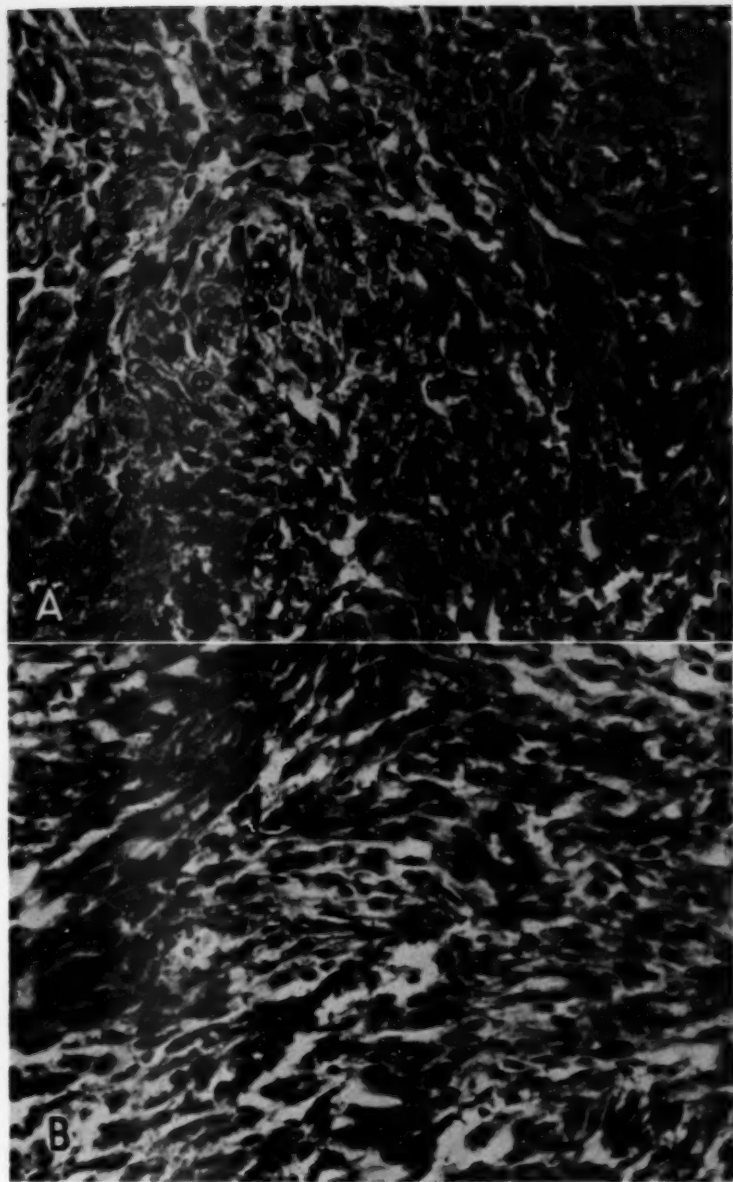


Fig. 1.—*A*, whorled meningeomatoid field from an intracranial pigmented tumor of the meninges; relatively little pigment is seen. *B*, fasciculated arrangement of the cells; more pigment is visible. Masson-Goldner stain; $\times 280$.

and there is a small amount of pigment in the normal meninges. That melanin is produced by fibrocytes or connective tissue elements is no longer generally accepted. An extensive list of references on this subject as well as an excellent discussion of the matter will be found in Laidlaw's¹⁹ article on studies of melanoma.

To stress this point, 2 cases that recently came under observation in the New York Hospital might be profitably mentioned; they are reported in extenso elsewhere (Ray and Foot²⁰). The first tumor was of a type that might fall in Cushing and Eisenhardt's "type I, variant 2 or 3" had it not been pigmented. Figure 1 *A* illustrates a typical "meningiomatous" field from this tumor, which occurred in the cranium near the pontile angle. Its cells were in every way like meningocytes, even to the rounded bodies and bulbous processes noted in the tissue cultures of Buckley and Eisenhardt. They formed whorls or fascicles (fig. 1 *B*) of cells in the typically meningiomatic fashion. Between these in the looser spaces of the stroma were collections of deeply pigmented pleomorphic cells, resembling those of a malignant melanoma. Silver impregnations revealed a copious reticular stroma, which might make the tumor more like type II. Mitotic figures were not found. The tumor invaded the adjacent occipital bone exactly as does the osteoplastic meningioma, an example of which my co-workers and I have recently studied (fig. 2 *B*). The cells in the bone spaces were pigmented, fairly orderly in their arrangement and less pleomorphic than those in the main tumor. The patient is still alive and free from symptoms well over a year after operation.

The second tumor (fig. 3 *A*) was very similar to the first except that it arose in the spinal canal and showed a less densely packed type of architecture. Had it not been pigmented it would have fitted into Cushing and Eisenhardt's type I, variant 3, or possibly type V. It was, however, densely pigmented. There were areas where there were whorls in this tumor, and many branching bundles of cells were also seen. Reticulum was present only in the neighborhood of the vessels. From its deeply pigmented and pleomorphic character, one would at first have called it a malignant melanoma. No mitoses were found in it, however, and the patient is alive and well nearly five years after operation, which would scarcely be the case had this been a metastatic or even a primary malignant melanoma. Figure 3 *B* shows a field from an ordinary meningioma for comparison with these 2 tumors.

19. Laidlaw, G. F.: *Am. J. Path.* 8:477, 1932.

20. Ray, B. S., and Foot, N. C.: *Primary Melanotic Tumors of the Meninges: Resemblance to Meningiomas*, *Arch. Neurol. & Psychiat.*, to be published.

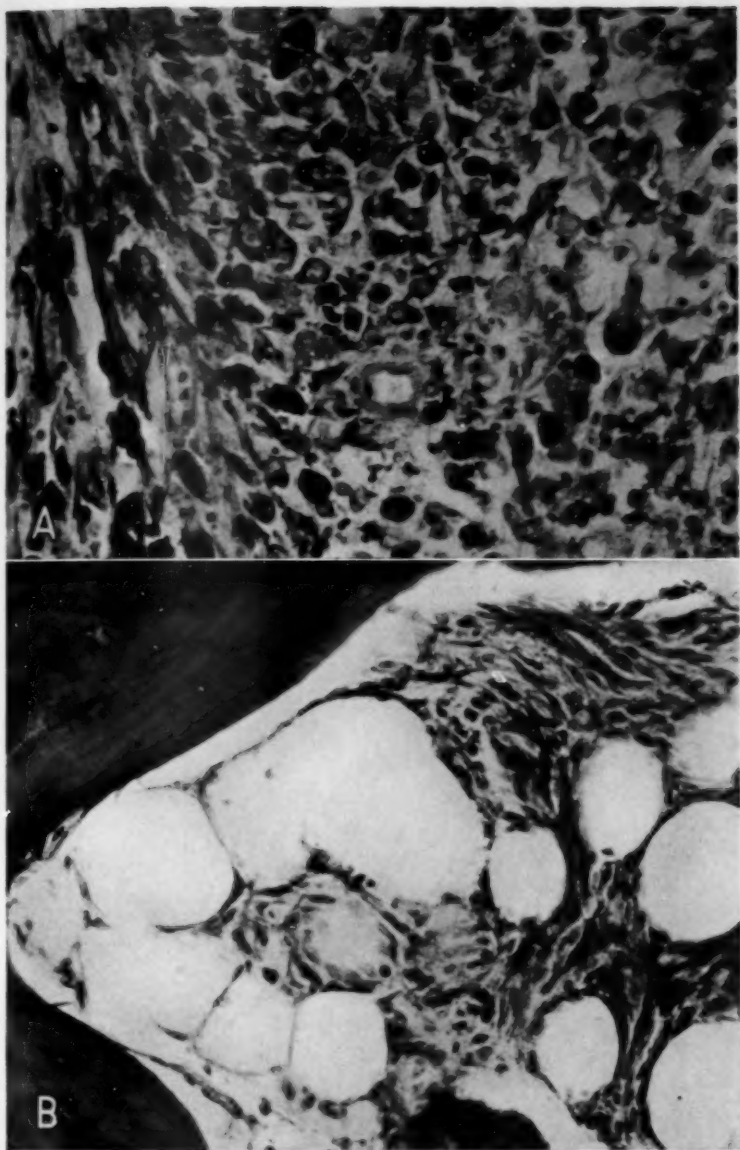


Fig. 2.—*A*, deeply pigmented, loosely arranged cells in stroma of the same tumor. *B*, tumor invading marrow of occipital bone. Note the relatively innocuous appearance of the cells, which are nevertheless pigmented. Masson-Goldner stain; $\times 280$.

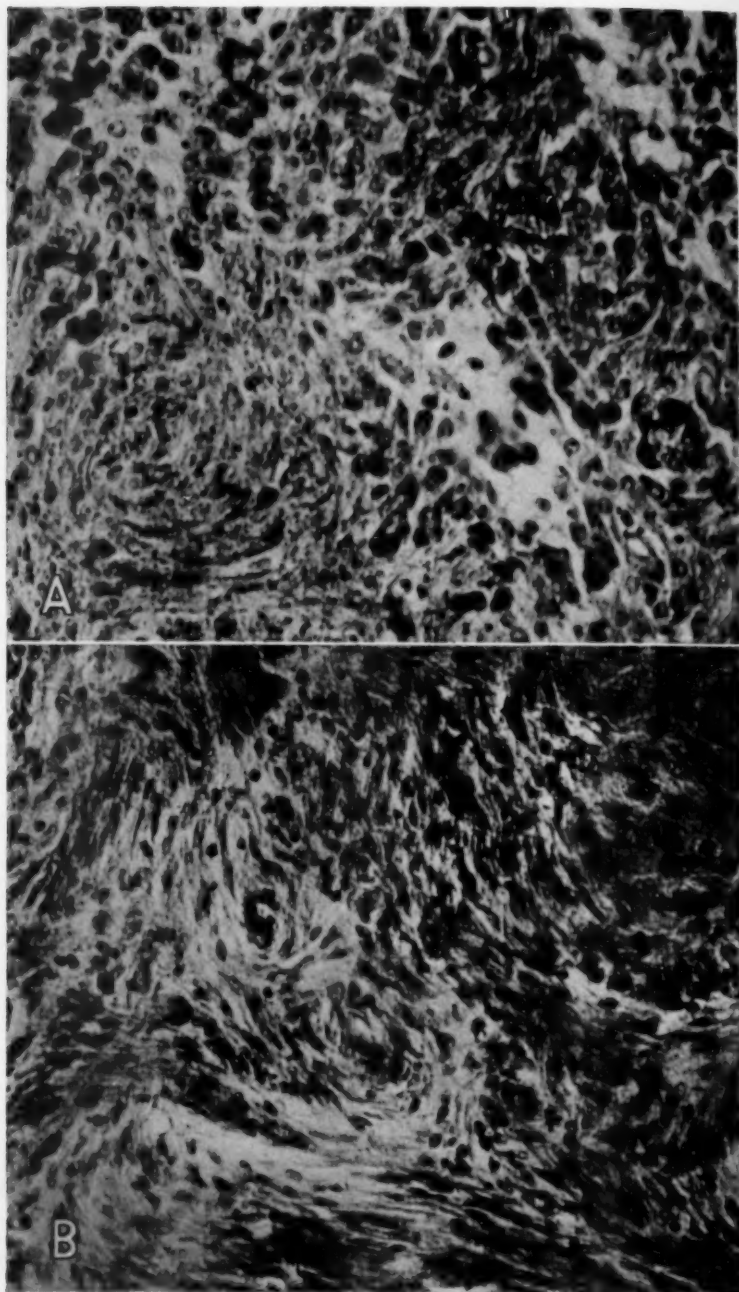


Fig. 3.—*A*, the same whorled arrangement is noted in the intraspinal tumor, and the pigmentation is evident. *B*, whorled and fasciculated fields from a non-pigmented meningioma, for comparison. Masson-Goldner stain; $\times 280$.

Melanoma of the cord or brain is rare, Schnitker and Ayer²¹ having collected only 29 instances from the literature and added 1 to the list. Two of their reported instances were observed by Zeek and myself (Foot and Zeek²²). This series covers eighty years since Virchow reported the first case. The tumors are usually situated where pigmented cells may normally be found; they are seldom metastatic, only 3 of the 30 collected showing metastasis outside of the nervous system—one to the lung, the other to the liver and spleen. A full discussion will be found in the article of Schnitker and Ayer,²¹ together with a good list of references. The tumors have been supposed to originate in terminal sensory organs in the meninges, structures that are extraordinarily inconspicuous. "Pigmented meningeal cells" are also mentioned as possible sources. These tumors may spread so solidly over the meninges as to coat the brain and cord with a layer of nearly black tissue a few millimeters in thickness. It seems unlikely that terminal organs would give rise to so diffuse a spread; it is more understandable that pigmented meningocytes might do so.

While one hesitates to say that these tumors are examples of the pigmented meningioma and hence differ from the usual melanoma, the impulse to do so is strong. If one ignores the pigment, the architecture of the 2 tumors is identical, as may be seen from the illustrations of this article. Masson²³ has soundly established the theory that the systemic melanoma arises from a perverted growth of Meissner's corpuscles. In the nonmalignant members of the group it is usually easy to see an analogy between the cell nests and disarranged tactile corpuscles; in the malignant forms, however, this is not so evident. Zeek and I were convinced that we had found such an analogy in our reported cases, but in the light of the 2 cases just described it is possible that we were dealing with a malignant variety of the pigmented meningioma rather than with a true melanoma. To explain those meningiomatoid tumors that do not show malignancy, then, would require a good deal of argument concerning the metaplasia away from the typical architecture of the Meissner corpuscle. If, on the other hand, one considers them to be pigmented meningioma, no extensive argument is necessary beyond accounting for the presence of pigment.

If one examines ordinary meningiomas that have been fixed in Zenker's fluid, one will note many refractile cells that have a darker, yellower cast than their fellows. Following this lead, if one impregnates such tumors by one of the Cajal silver nitrate methods, one will find that such cells contain argentaffin granules that are rather conspicuous

21. Schnitker, M. T., and Ayer, D.: *J. Nerv. & Ment. Dis.* **87**:45, 1938.

22. Foot, N. C., and Zeek, P.: *Am. J. Path.* **7**:605, 1931.

23. Masson, P.: *Ann. d'anat. path.* **3**:417 and 657, 1926.

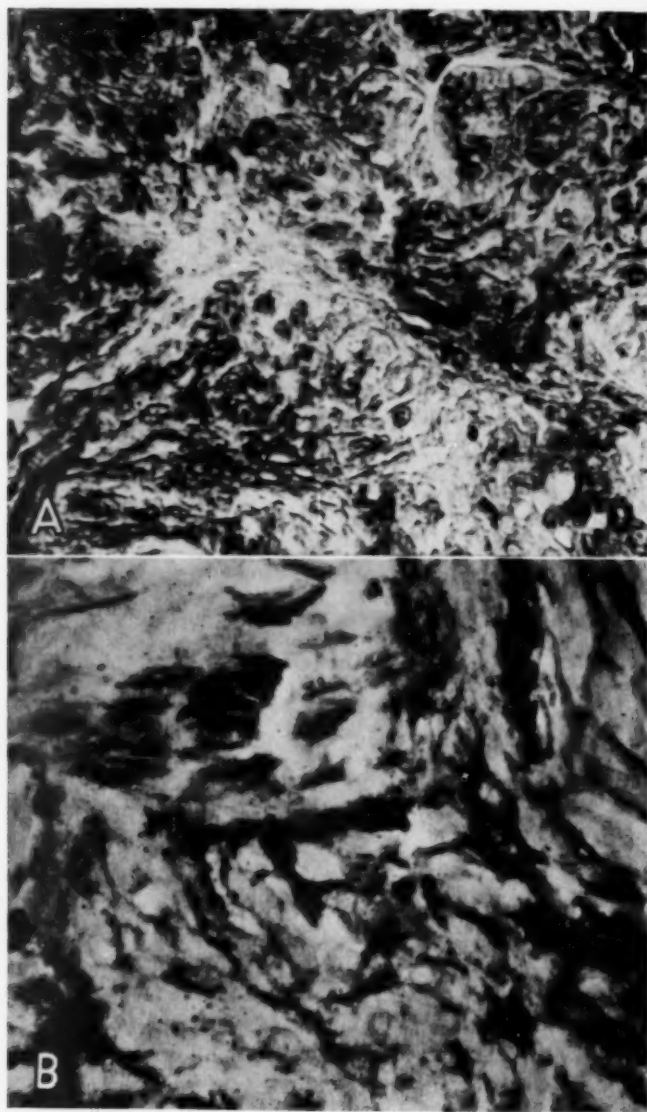


Fig. 4.—*A*, Cajal impregnation of a supposedly nonpigmented osteoplastic meningioma. Note the argyrophil granules near the center of the picture, some black, some grayish. The latter may be premelanin. $\times 550$. *B*, Cajal impregnation of the intracranial pigmented meningioma. Note the abundance of argyrophil granules and the cells packed with them. Again, the paler granules may represent premelanin. $\times 550$.

and probably represent premelanin. Figure 4 *A* shows such a section from a nonpigmented osteoplastic meningioma that will be mentioned later; figure 4 *B* shows one from the intracranial pigmented meningioma just described. It will be seen that there are very few but nevertheless distinct argyrophil granules in the first and large numbers in the second, sometimes filling the cells to capacity. This would apparently indicate that meningocytes do contain a small quantity of melanin or premelanin.

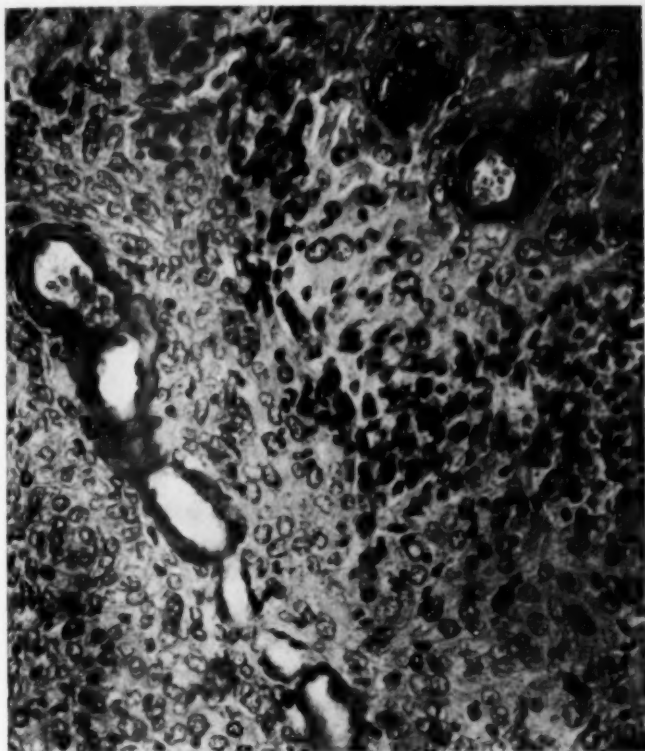


Fig. 5.—Masson-Goldner preparation of a section from the osteoplastic meningioma. The smaller, darker cells contain the granules that are demonstrable with the silver method (see fig. 4 *A*). $\times 280$.

If one is to base an explanation of the presence of such pigment on an elaboration of melanin by fibrocytes or derivatives thereof, one will encounter difficulties and be forced to reopen the question that has so long been successfully disputed by Masson and apparently settled in his favor.

Cushing and Eisenhardt collected 6 examples of the interesting osteoplastic meningioma in their series of 306 meningiomas. My co-workers and I have had one (fig. 5) at this hospital, which presents some features

that may have a bearing on its histogenesis. Like the pigmented tumor that invaded bone, this one tended to lose its marked pleomorphism once it lay in the bone spaces, where its cells became smaller and better differentiated. The main tumor was quite lawless and showed numerous mitotic figures, but these were absent in the invasive growth. It was definitely epithelioid in appearance (in the primary site), and its cells showed an affinity for orange G in the Masson-Goldner sections. Cells that do this are usually associated with some sort of keratin. Those of the stratum corneum of the epidermis are especially prone to take up the orange stain. This is true of the nerve sheaths containing myelin, where there are abundant Schwann cells. Fibrocytes do not take it up.

Having touched on the subject of malignant meningioma in the preceding paragraphs, I come to a final note on the natural history of meningioma. It is often noted that tumors undergoing malignant change, or malignant from the beginning, tend to recapitulate the embryologic development of their type cell. This is what is called anaplasia. Recently Bailey and Cushing²⁴ constructed a classification of tumors of the glioma group on such a recapitulation plus recourse to the embryology of the nervous system. The meningiomas show malignant varieties, of which there are two general types: those that imitate the alveolar, epithelioid meningioma and those that are frankly of connective tissue origin, fibrous or retothelial. The former are the more frequently encountered, and one sees, therefore, more numerous epithelioid examples and fewer that arise in connective tissue.

If this larger group were derived from fibroblasts, one would expect them to recapitulate their development and form giant fibroblasts or spindle cells as they do elsewhere in the body when fibrosarcoma develops. This is not the case.

Before concluding it might be well to say a few words concerning classification. It will be seen that that of Cushing and Eisenhardt essentially expresses the ratios between parenchyma and stroma in the meningiomas, with various subtypes to indicate angioplastic, osteoplastic or other such tendencies. In their series the "parenchymatous" types outweigh the stromal in their number (nearly 200 of 306). The series collected at our hospital during the past seven years numbers only 16, of which 8 are the meningocytic, 2 the fibrous psammomatous, 4 the angioplastic and 1 each the reticulosarcomatous and the osteoplastic type. Our 2 pigmented tumors are not included in this series, pending a time when their nature is more definitely decided on. This small series

24. Bailey, P., and Cushing, H.: *A Classification of the Tumors of the Glioma Group on a Histogenetic Basis with a Correlated Study of Prognosis*, Philadelphia, J. B. Lippincott Company, 1926.

bears out the percentages of the much larger New Haven series in a general way, although the angioplastic representatives are at present a bit more numerous.

There is a rather striking analogy in such a series to one composed of, say, mammary carcinomas. There are the largely epithelial carcinomas, intermediate types and those with an overgrowing stroma (scirrhous) at the other end of the scale. In all of them, however, the growth stimulus apparently resides in the epithelial cells. Might this not be possible in the meningiomas also? The type cell would, then, be the "meningoblast" of Oberling or the "meningocyte" of Cushing. It is doubtful that a better term for these cells could be found at present, as there is nothing to express their status unless one coins the word "neurothelium" for cells of probable neural crest origin. This is inadvisable until their neural crest parentage is definitely accepted.

SUMMARY

To sum up, then, the meningioma appears to be a tumor of composite makeup, comprising two groups of cells: the meningocytes, derived from the arachnoid villi, and the stroma cells, coming from the mesenchymal elements of the pia-arachnoid. Usually the former predominate, and it is probable that they represent the type cell of the meningioma.

The weight of experimental evidence points toward derivation of these cells from the neural crests, and this view is reenforced by their dissimilarity to fibroblasts both in respect to their morphologic aspects and in respect to their staining reactions with acid fuchsin, ponceau de xylidine and orange G, for all of which they show an affinity not evidenced by fibroblasts. From the standpoint of their architecture they more closely resemble growths associated with neural than those associated with pure fibrous tissue. The importance of the presence of melanin in members of the same series should be stressed and a relationship between pigmented meningeal tumors and meningiomas tentatively advanced. The production of psammoma bodies, or corpora arenacea, has purposely been ignored in this paper, as they appear to be by-products which are normally seen in pacchionian bodies and may occur in other tumors, where they appear to be definitely subsidiary products. It is also to be noted that the malignant melanomas usually tend to have an epithelioid rather than a fusocellular sarcomatoid appearance such as might be expected from derivatives of fibroblasts. It is hoped that this article may be of use in evaluating the literature on meningioma and may clear up some points that might be confusing.

IMMUNITY TO FOWLPOX STUDIED BY MEANS OF
SKIN GRAFTS ON CHORIOALLANTOIS
OF CHICK EMBRYO

E. W. GOODPASTURE, M.D.
AND
KATHERINE ANDERSON, Ph.D.
NASHVILLE, TENN.

The mechanism of acquired immunity to virus infections is as yet but poorly understood. Although after recovery from any one of several diseases of this type the host becomes apparently completely resistant to reinfection and the blood serum acquires the capacity to neutralize the respective virus, there is still a question whether or not the susceptible cells themselves participate in this immunity.¹ There is little direct evidence on this point, and some investigators are reluctant to attribute the acquired refractory state solely to humoral factors.

Acquired immunity to fowlpox has offered especial difficulties to an acceptable explanation of the immune state because there is inadequate evidence respecting the presence in the recovered host of humoral antibodies to the virus and no direct evidence that the normally susceptible epithelial cells themselves acquire resistant properties.²

This disease of birds, fowlpox, is of especial interest in connection with the problem of cellular immunity, also for the reason that there is experimental proof that the virus enters the susceptible cells, multiplies within them and becomes a constituent of the specific cellular inclusions (Bollinger bodies) of the characteristic lesion.³

Notwithstanding the lack of knowledge regarding the cellular immunity following fowlpox, this disease offers an exceptionally favorable opportunity for experimental study of certain phases of this problem because the virus affects especially cutaneous epithelium, manifesting itself by easily recognizable specific inclusions, and the skin of both the

From the Department of Pathology, Vanderbilt University Medical School.

This investigation was aided by grants from the John and Mary R. Markle Foundation and the Division of Medical Sciences of the Rockefeller Foundation.

1. Rivers, T. M.: *Lane Medical Lectures: Viruses and Virus Diseases*, Stanford University Publications, University Series, Medical Sciences, Stanford University, Calif., Stanford University Press, 1939, vol. 4, no. 1, p. 53.

2. Goodpasture, E. W., and Rivers, T. M.: *Virus Diseases*, Baltimore, Williams & Wilkins Company, 1928.

3. Woodruff, C. E., and Goodpasture, E. W.: *Am. J. Path.* **5**:1, 1929; **6**:713, 1930.

normal and the immune fowl can readily be grafted onto the chorio-allantois of the chick embryo.⁴ The latter fact affords a favorable method for studying the behavior of cutaneous epithelium under different environmental circumstances.

Somewhat similar, although in vitro, methods have been used in experimental investigations of immunity to certain other virus infections.

Steinhardt and Lambert, in 1914, were the first to test the susceptibility of tissues from normal and immune animals in vitro by planting small pieces of corneal epithelium in plasma.⁵ They used vaccinia virus and found that corneal epithelium from an immune rabbit completely resisted infection under the conditions of their experiment, although normal corneal epithelium supported multiplication of the virus.

Similar technics have been used by several later investigators to test the susceptibility of tissue transplants to a variety of viruses. Rivers, Haagen and Muckenfuss,⁶ by implanting pieces of rabbit cornea onto clotted plasma, found that normal cornea inoculated in vitro with vaccine virus and cultivated in antivaccinial plasma clot presented typical vaccinial lesions, associated with active virus, while immune cornea similarly inoculated and cultivated in either normal or antivaccinial plasma clot revealed very mild lesions or none. Active virus was demonstrated only in a portion of the latter preparations.⁶

Andrewes, working with virus III and surviving transplants of rabbit testis in normal and immune plasma, found that this virus would survive and form specific inclusions in cultures of immune testis in normal serum or plasma, particularly if the immune tissues were subjected to brief preliminary soaking in Tyrode's solution.⁷ From his experiments Andrewes drew the conclusion that protection of susceptible cells from infection by virus III depends on the antibody content of the fluids surrounding the cell and not on any active immunity of the attacked cells.

Later investigations, by means of tissue culture or survival technics in vitro, with these and other viruses have not led to uniform conclusions respecting the problem of active cellular immunity. For example, Topacio and Hyde concluded, because in their experience immune plasma inhibited the action of virus III on cultures of normal rabbit testis, and because immune testicular tissue was not infected even after washing in Tyrode's solution, that the immunity resulting from virus III infection is of both the cellular and the humoral type.⁸

4. Goodpasture, E. W.; Douglas, B., and Anderson, K.: *J. Exper. Med.* **68**:891, 1938.

5. Steinhardt, E., and Lambert, R. A.: *J. Infect. Dis.* **14**:87, 1914.

6. Rivers, T. M.; Haagen, E., and Muckenfuss, R. S.: *J. Exper. Med.* **50**:665, 1929.

7. Andrewes, C. H.: *Brit. J. Exper. Path.* **10**:273, 1929.

8. Topacio, T., and Hyde, R. R.: *Am. J. Hyg.* **15**:99, 1932.

Andrewes, in pointing out the advantage of the tissue culture technic in an experimental approach to the problem of immunity, stated that such an analysis was not possible by *in vivo* methods.

Our success, however, in readily establishing skin grafts on the chorioallantoic membranes of developing chick embryos led us to undertake a study of immunity to fowlpox by the use of this *in vivo* technic. Our first objective was to determine whether or not established skin grafts from normal and immune fowls were susceptible to infection with the virus of fowlpox, as manifested by the development of the pathognomonic Bollinger bodies within the cytoplasm of the epithelial cells following inoculation. We have also transplanted skin grafts from chorioallantois back to the original or another fowl.

Several experiments have also been carried out to test the *in vitro* and *in vivo* effects of serum, plasma and whole blood of immune chickens on the virus of fowlpox.

TECHNIC

Six adult domestic cocks were infected by plucking feathers from the breast and rubbing a suspension of fowlpox virus into the injured follicles. Typical lesions of fowlpox resulted. After the skin healed, reinoculations were made at intervals until complete cutaneous immunity had been established in a period of about four months after the initial inoculation.

After unfeathered areas of skin over the breast were washed with soap and water, rinsed with water, dried, painted with iodine, washed immediately with alcohol and again dried, thin pieces of skin from the unfeathered portions of the breasts of normal and of immune cocks were dissected off by splitting the corium with a sharp scalpel. This was best achieved by making a triangular incision through the epidermis of the skin, picking up the apex with small mouse-toothed forceps and dissecting off the thin superficial layer. The skin thus removed was placed on a moist sterile cork board and cut into pieces measuring at least 0.5 cm. square. These were spread out, corium down, onto the freshly exposed chorioallantoic membranes of chick embryos 11 days old. The grafts were usually allowed to remain three or four days before inoculations were made. Inoculation was accomplished by snipping the surface of each graft with sharp-pointed iris scissors and rubbing over it, and sometimes leaving on it, a fragment of bacterially sterile virus-infected ectoderm of a membrane that had been inoculated with fowlpox virus ninety-six or more hours earlier.

It was our practice to wait ninety-six hours after inoculating the grafts before removing them for histologic examination, because it takes that long a period for full development of the specific inclusions, *i. e.*, the Bollinger bodies. At ninety-six hours the grafts were removed by cutting out the membranes to which they were attached. They were then fixed in Zenker's fluid (5 per cent acetic acid). Paraffin sections were stained with hematoxylin and eosin. The cytoplasmic inclusions within hyperplastic epithelium stain a bright red and are pathognomonic of the infection. The grafts were, as a rule, well established by the time they were removed, and extensively vascularized. The infected epithelium was swollen and hyperplastic.

For purposes of control, normal skin was grafted in the same way, often onto the same membrane with skin from an immune bird. In this way a com-

parison could be made of the two grafts side by side in the same section and from the same embryo.

SKIN GRAFTS ON THE CHORIOALLANTOIS

In collaboration with Dr. Beverly Douglas, we recently published our experiments in grafting human skin onto the chorioallantois.⁴ We found that thin grafts of human skin implanted on the chorioallantois adhered and were nourished for as long as ten days, representing the full period of subsequent development of the embryo. The epithelium of the chorioallantois fused with that of the graft; the collagen fibers of the corium interlaced with those of the membrane; the blood vessels anastomosed and united, by intervening pools of extravasated blood, with those of the graft. The grafts were nourished especially by a plasmatic circulation, although there was gradual revascularization by ingrowth of blood vessels from the chick membrane.

Chicken skin is better adapted for membranal grafting than human skin. Thin sheets of epidermis can be easily obtained, infection rarely interferes, and the grafts become rapidly revascularized. Grafts implanted on the membranes of 11 day old embryos have a period of eight to ten days during which observations can be made. During this period the membranes are very susceptible to infection, following inoculation, by the viruses of fowlpox and vaccinia.

If adjacent edges of two grafts are closely approximated, they do not unite, in our experience, although frequently superficial cuts in the graft will heal.

FOWLPOX INFECTION OF SKIN GRAFTS ON CHORIO-ALLANTOIC MEMBRANES

Normal Skin Grafts.—The epithelium of the chorioallantois is susceptible to fowlpox virus, and when the grafts are in process of being inoculated the contiguous epithelial cells also become infected. This is evidenced grossly after about forty-eight hours by slight thickening of the membrane adjacent to the graft. At ninety-six hours this thickening, together with some wrinkling of the membrane, is conspicuous. It is necessary, however, to examine the graft in microscopic sections to be certain that infection of the cutaneous epithelium has taken place.

The first evidences of fowlpox infection are swelling of epithelial cells and hyperplasia of the basal layer. These are to be seen after about forty-eight hours. At this time a few vacuoles, representing the earliest and most fatty forms of the specific inclusions, appear. At seventy-two hours the swelling and hyperplasia are more marked, and more well defined inclusions, some of which stain red with eosin, can be seen; but the ninety-six hour period is best for an easily interpretable picture of the infection, for at that time great numbers of infected cells

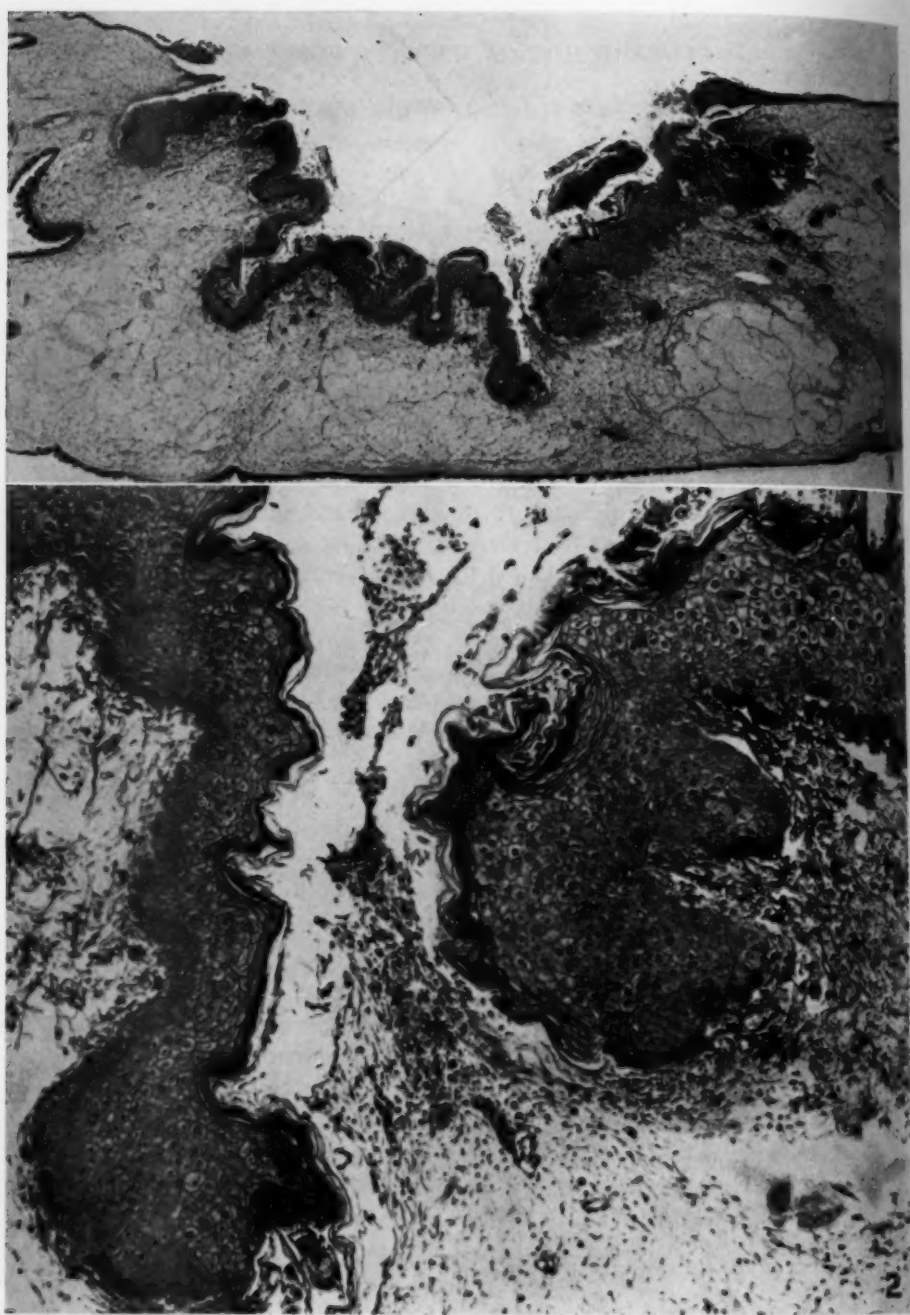


Fig. 1.—1, normal (left) and immune (right) chicken skin grafted on the same chorioallantoic membrane; $\times 25$. Each was inoculated four days after grafting, and each was removed for section four days after inoculation.

2, the ends of the two grafts shown in figure 1, at the center; $\times 150$. The epithelial cells of both the normal (left) and the immune (right) grafts show abundant Bollinger bodies (dark masses) in the cytoplasm. The immune skin is quite as susceptible as the normal.

contain large eosin-staining cytoplasmic inclusions that are specific. By that time also hyperplasia and swelling of epithelium are considerably advanced. There is no inflammatory reaction in the graft that is with certainty attributable to the infection.

The ninety-six hour interval after inoculation was therefore adhered to in these experiments as the minimum period of incubation before grafts would be removed for microscopic section. In certain experiments longer periods were allowed.

Whether the inoculations were made immediately after grafting or at varying intervals there was no evident difference in the susceptibility of the epithelium as indicated by the ninety-six hour lesions. We usually allowed the graft to remain on the membrane three or more days before inoculation, so that it might become well attached and nourished.

Immune Skin Grafts.—As a preliminary control for further experiments several pieces of skin from immune chickens were grafted on the chorioallantois and allowed to remain without inoculation to determine whether or not fowlpox infection would manifest itself under these conditions from a latent virus infection of this tissue or from virus contamination of the skin. In no instance was there any indication of fowlpox infection in these grafts. This is of some interest in view of the fact that immune skin was found to be susceptible to infection with fowlpox virus after implantation on the chorioallantois; therefore it is assumed that had latent virus been present in the skin it would have manifested itself by observable changes.

Other pieces of skin from immune chickens were inoculated after varying lengths of time following implantation on the chorioallantois. Some were inoculated by snipping the ectoderm with scissors and rubbing over it a piece of infected membrane two hours after, or at intervals up to four and five days after, grafting. Ninety-six hours after inoculation the pieces were removed and examined histologically. All showed active fowlpox infection, indicated by the usual characteristic epithelial changes, including, of course, the presence of typical Bollinger bodies.

In several instances a graft of immune skin was implanted beside a graft of normal skin. Inoculations were made simultaneously, and after ninety-six hours the two specimens were removed in one piece and sectioned. In these preparations, as in single ones, no recognizable differences in susceptibility between the normal and the immune grafts could be detected.

Immune and Normal Skin That Had Been Inoculated Before Removal for Grafting.—This experiment was made to determine whether immunity would manifest itself by an absence of infection following grafting if the skin were inoculated while it remained a part of the immune host. The skin was washed and treated with iodine and alcohol

EXPLANATION OF FIGURE 2

1, normal chicken skin that was grafted four days onto normal chicken (autograft), inoculated with fowlpox and removed four days later; $\times 225$. Note large Bollinger bodies in epithelial cells.

2, immune chicken that was grafted three days on chorioallantois, removed and grafted four days on muscle of normal chicken, inoculated with fowlpox and removed four days later; $\times 225$. Note infection as indicated by Bollinger bodies in epithelial cells.

3, immune chicken skin that was grafted four days on chorioallantois, removed and grafted on immune chicken (autograft) four days, inoculated with fowlpox virus and removed four days later; $\times 46$. Note absence of any evidence of epithelial infection.

4, scalp of chick that received 1 cc. of normal whole chicken blood intravenously and was then inoculated locally with fowlpox virus; $\times 25$. This six day lesion shows extensive infection and edema. This was the control for 5.

5, scalp of chick that received 1 cc. of whole blood from an immune fowl and was then inoculated after plucking the down from the scalp; $\times 25$. Although the infection is just as extensive as in 4, the lesion is only one half as thick, and there is much less edema.

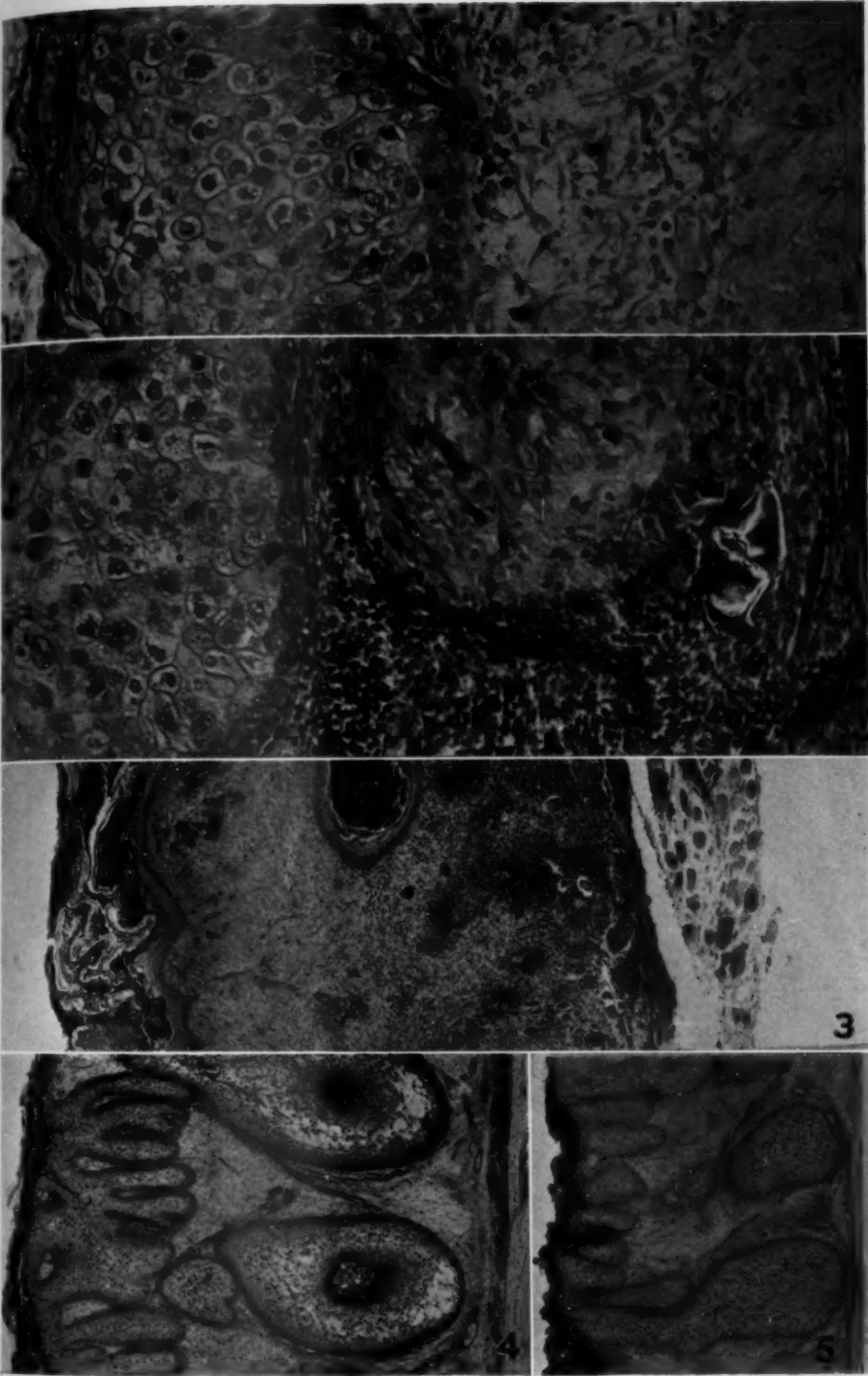


Figure 2
219

in the usual way. It was then lightly scarified and covered with a suspension of virus which was allowed to dry for fifteen minutes. The scarified epidermis was then removed in the usual way and pieces grafted on embryos. All 6 of these grafts showed infection after ninety-six hours.

Another experiment, a much more drastic one, was made by scarifying and inoculating the skin before cleansing it. The skin was inoculated by applying to the scarified area a thin paste of ground infected membrane. This was allowed to remain for thirty minutes. The scarified and inoculated skin was then washed thoroughly with soap and water; iodine was applied and immediately removed with alcohol. After drying, the inoculated epidermis was dissected off and pieces grafted on embryos. Two embryos were killed at each interval of four, five, six and nine days. In one of the grafts removed from the embryos killed after nine days infection was observed. The others showed no infection. Owing to the uncontrollable and severe features of this treatment, it is not possible to interpret the negative results.

EFFECT OF REGRAFTING OF CHORIOALLANTOIC SKIN GRAFTS
ONTO MUSCLE AND DENUDED CORIUM OF THE
SAME AND DIFFERENT BIRDS

Immune Skin Regrafted onto Same Birds.—The susceptibility of chorioallantoic grafts of skin from immune fowls raised the question whether the skin surviving in this abnormal environment and nourished by embryonic fluids was positively altered in some way that rendered it susceptible, or whether an innate susceptibility on being removed from inhibiting influences of the immune host could manifest itself under these conditions.

If the latter hypothesis was true, the susceptible grafts being removed from the chorioallantois and regrafted on an immune host would again become insusceptible.

Grafts from an immune cock were therefore implanted on an embryo, and after three and four days they were removed and regrafted on muscle and denuded corium of the same bird.

For regrafting on muscle an incision was made through the skin of unfeathered areas of the breast down to the fascia overlying the pectoral muscles. The fascia was then incised and dissected away from the underlying muscle on either side for about 1 cm. The pieces of skin were then placed on a flat spatula and inserted between fascia and muscle, with the corium of the graft overlying the raw muscle. In this way the overlying fascia served as a protecting splint for the graft. The skin incision was either left open or was closed with two or three silk sutures. The grafts "took" readily as a rule, and after

three or four days the wound was reopened, the pieces of skin were exposed by lifting up the fascia, and the epidermis was inoculated by rubbing over it a piece of infected membrane, after snipping the surface with iris scissors. The grafts were removed four days later for microscopic study. Six experiments were performed, and no grafts showed evidence of infection.

In 2 instances successful regrafts were obtained by placing grafts on raw corium after denuding as in obtaining skin for original grafts. About a third of such grafts survived four days. After implantation they were greased each day with petrolatum. No other dressing or treatment was applied. These regrafts, as well as those on muscle, proved to be completely resistant to infection.

Normal Skin Grafted onto Normal and onto Immune Fowls.—In order to control the experiments just described by determining whether or not normal skin grafted onto the chorioallantois retained its susceptibility when regrafted on the normal fowl from which it was obtained, the same procedure was carried out in a new set of experiments. The grafts were removed from the chorioallantois three days after implantation. They stripped off easily and were regrafted on muscle as indicated. Grafts from a normal chicken were regrafted on the muscle of the same fowl and on that of an immune fowl, 2 grafts to each bird. Four days later they were exposed and found to be attached and viable. They were snipped on the surface with iris scissors and inoculated with pieces of infected membrane, which were allowed to remain in contact with each graft. Four days later they were removed and fixed. The 2 autografts each showed a well developed fowlpox lesion, while both those from the immune bird, although viable, presented no evidence of infection.

ATTEMPTS TO DEMONSTRATE HUMORAL ANTIVIRAL EFFECTS

In none of our experiments with skin grafts did there appear any evidence that epithelial cells had an acquired immunity to fowlpox virus after their removal from the immune host. On the other hand, immunity reappeared when susceptible skin grafts were reimplanted successfully on the immune host. This fact pointed strongly to a humoral antibody mechanism of resistance to fowlpox. We therefore made attempts to demonstrate the activity of such a mechanism.

In all of our experiments we purposely refrained from using hyperimmunized animals because we wished to avoid any factors that might be introduced by parenteral injections of virus and perhaps other antigens. Our experimental animals were immunized only by cutaneous inoculation after scarification; and the immunity was perhaps enhanced by the necessary reinoculations to assure complete cutaneous resistance.

The strain of virus used for the original inoculation induced a mild local lesion, and general immunity developed rather slowly. This strain of virus had been maintained for two years in the chorioallantois without bacterial infection, and the lesions were almost completely epithelial in character.

Attempts to Immunize Embryos Passively With and Without Skin Grafts by Intravenous Injection of Serum from Immune Birds.—Serum from an immune bird was injected in varying amounts up to 0.2 cc. into the chorioallantoic veins of several embryos. Within one hour the chorioallantoic ectoderm was inoculated with fowlpox virus. In due time a well developed infection appeared.

Two other groups of embryos received immune serum intravenously. On one group normal chicken skin, and on the other group immune chicken skin, was grafted in the usual manner immediately after the injection. After seventy-two hours the grafts were inoculated with fowlpox virus after the epidermis had been snipped with iris scissors. Ninety-six hours later the grafts were removed for sectioning, and all were found to be heavily infected. This experiment was repeated with minor variations and with the same result.

There was no evidence, therefore, that an effective passive immunity, either for the chorioallantois or for normal and immune skin grafts on it, could be conferred by intravenous injection of 0.2 cc. of serum from immune fowls. It did not appear that skin grafts from immune fowls differed in this respect essentially from those from normal chickens.

Larger amounts of serum could not be injected into embryos with safety.

Attempts to Immunize Newly Hatched Chicks Passively by Injections of Serum, Plasma, Whole Blood and Leukocytes.—Embryos would not withstand intravenous injection of more than 0.2 cc. of serum. In order to test the effect of higher concentrations, we resorted to the use of baby chicks from 2 to 7 days of age. These chicks weighed approximately 60 Gm.; they would easily withstand intravenous injections of 1 cc., and in certain instances a total of 3 cc. of whole blood was injected in 1 cc. amounts on successive days without apparent ill effects. The wing veins were used for the injections.

For the test inoculation the down over the crown of the head was plucked, and the virus suspension was applied to this area with a camel's hair brush without further injury to the skin, using a uniform number of strokes, with the brush saturated with the virus suspension. Infection developed first in the follicles and later in the intervening skin, or in both simultaneously.

In brief, there was little evidence that serum or heparinized plasma from immune chickens injected intravenously in 1 cc. amounts exerted any appreciable effect on the extent or the quality of the infection.

On the other hand, the same volume of whole heparinized blood or of heparinized plasma and leukocytes caused a moderate reduction in the amount of infection and in the character of the lesion. Whereas the controls, receiving the same amount of normal plasma or of plasma and leukocytes, showed on the fourth day an infection which involved the skin of the entire inoculated area, associated with a great deal of edema, the chicks that received immune whole blood or plasma and leukocytes presented numerous discrete focal lesions without edema, in experiments in which suitable dilutions of virus were used for the test inoculations.

These experiments were repeated several times, with uniform results. Total counts of infected foci on the scalp in suitable experiments showed about 10 per cent less foci in the passively immunized chicks as compared with the controls.

More striking than the numerical differences in the extent of infection was the contrast in the quality of the lesion. In those experiments in which the inoculating dose was sufficient to cause a completely confluent lesion in both groups, the normal chicks and those that received normal blood or normal plasma and leukocytes showed a cutaneous edema which, together with the thickening of the infected epithelium, caused the skin to become almost twice as thick as that of the passively immunized.

It seemed to us that an injurious substance was released by the infected skin which induced the edema, and we concluded that the blood and the plasma-leukocytes from immune chickens served to neutralize this hypothetical agent. On the other hand, despite the fact that the epithelium of both follicles and skin in each group were uniformly infected, the number of infected cells in the control groups was greater than the number in the groups passively immunized. It is possible, therefore, that the edema was an expression of the number of cells involved rather than an evidence of lack of neutralization of an edema-inducing agent. This phenomenon will be considered in the discussion.

No satisfactory explanation is at hand for the difference in effectiveness of immune whole blood and plasma-leukocytes and of immune serum and plasma alone.

Attempts to Demonstrate Passive Immunity by Intravenous Injections of Immune Blood, Serum and Virus.—Because of failure to demonstrate clearly passive immunization by means of intravenous injections of immune blood followed by inoculation of the plucked scalp, we undertook to determine whether or not this might be more effectively brought to light by injecting both immune blood and virus intravenously, subsequently injuring the skin by plucking down from the scalp and observing secondary localization of lesions in that area.

Normal whole blood or serum (1 cc. each) was injected intravenously into 2 week old chicks. A few minutes later a suspension of fowlpox virus was similarly injected, and immediately afterward the down was plucked from the head. In one set of experiments a 1:20 virus sus-

pension was used and in another a 1:100 suspension. After four days well marked lesions had developed in the scalp in both series, less marked in those receiving the more dilute virus suspension.

In similar series of chicks of the same age that received immune whole blood or immune serum and the same dilutions of virus suspension, similar lesions developed in the scalp and in equal amount.

In this experiment, therefore, there was no evidence that, in the quantity used, either whole blood or serum from immune fowls protected chicks from the localization of circulating virus in, and subsequent infection of, an injured area of the scalp.

Attempts to Demonstrate Local Passive Immunity.—Inoculation of the plucked scalps of baby chicks following local cutaneous injections of serum, plasma and plasma-leukocytes from immune chickens resulted uniformly in the development of lesions indistinguishable from those in the controls that received similar normal fluids. The amount of immune serum or plasma injected cutaneously varied up to 1 cc. After the injection, the down was plucked, and the virus suspension was applied with a camel's hair brush.

Attempts to Neutralize Fowlpox Virus with Immune Serum and Whole Blood.—Although the injection of whole blood and plasma-leukocytes from immune chickens showed a definite but limited inhibitory effect on the test inoculatory lesions, these experiments were unsatisfactory from a quantitative standpoint, because even with the most dilute test suspensions of virus a few pox occurred both in the control and in the test groups, so that it was not possible for us to determine accurately quantitative relationships.

We therefore attempted to neutralize the virus in vitro with serum and whole blood from immune chickens. The results of these tests were similar to those which followed attempts at passive immunization. There was in several tests an average of about 10 per cent reduction in the number of pox in the chicks that were inoculated with virus incubated with immune serum and whole blood as compared with the controls.

The immune and the normal serums were incubated with various dilutions of virus for different periods of time and at different temperatures from two hours at 37 C. to six hours at 40 C.

COMMENT

These experiments conclusively proved that the epithelial cells of skin from chickens with acquired immunity to fowlpox, and completely resistant to infection by cutaneous inoculation while a part of the immunized host, become quite susceptible to infection by this virus after being grafted onto the chorioallantoic membranes of chick embryos. If such grafts, as well as grafts from normal chickens, were regrafted on muscle of immune chickens and inoculated, no infection took place.

Normal skin, on the other hand, when regrafted on the muscle of normal chickens was quite susceptible to infection by inoculation.

These experiments therefore gave no indication that acquired immunity was inherent in the epithelial cells themselves but pointed rather to humoral or local factors in other tissues as the cause of the acquired resistance of the normally susceptible epithelium.

Attempts to demonstrate an antiviral effect of immune serum, plasma and whole blood by *in vivo* tests of passive resistance and by *in vitro* tests involving incubation of these fluids with virus suspensions showed a very limited but rather distinct inhibitory action in a reduction of the quantity of pocks. This was more marked in the case of whole blood and of plasma plus leukocytes than in that of serum or plasma alone. In fact, the tests with serum and plasma alone were inconclusive.

In addition to modifying the number of pocks developing following inoculation of the skin, the intravenous injection of whole blood and of plasma plus leukocytes also modified markedly the quality of the lesion in that the infected skin failed to become edematous.

The development of extensive edema, involving both epithelial cells and corium, might be due to some substance released by the infected tissue, which was neutralized by the injected humoral antibodies; or it failed to occur in the passively immunized chicks because of the smaller number of cells that became infected following the procedure. In this connection it is considered that the swelling of epithelial cells adjacent to areas of infection might be a potent factor in the centrifugal spread of the virus from a local area by increasing the permeability of the cells and the exposure to the virus. The edema also might be instrumental in stimulating hyperplasia in such areas, thus affording more cells for contact with the virus.

We have at present no adequate explanation to offer for the greater potency of immune whole blood and of plasma-leukocyte mixtures as compared with serum in modifying the extent and character of inoculatory lesions. So far as they go, however, our experiments point rather to humoral antibodies as the most potent agents in the acquired immunity of cutaneous epithelium to fowlpox infection.

It seems likely that the antibodies to fowlpox virus occur in such low concentration that their demonstration by passive transfer and by *in vitro* neutralization is rendered difficult.

In view of the demonstration of the susceptibility of epithelial cells to fowlpox virus by the inoculation of skin grafts from immune birds implanted onto the chorioallantoic membranes of chick embryos, it seems likely that the relative resistance of tissues from hosts immune to certain other viruses (virus III, vaccinia) surviving *in vitro* is due to a higher concentration of antibodies in these tissues, which necessitates a longer period for their reduction to a critical or ineffectual level by diffusion and dilution.

LEAD ABSORPTION AND INTOXICATION IN MAN
UNASSOCIATED WITH OCCUPATIONS
OR INDUSTRIAL HAZARDS

ABSORPTION OF LEAD FROM ELEVEN WEEKS OF INTRAUTERINE
LIFE TO NINETY-THREE YEARS OF AGE

G. H. HANSMANN, M.D.

AND

M. C. PERRY, M.A.

MILWAUKEE

During the recent several years 4 cases of lead poisoning and 1 case in which lead perhaps was an important factor in the history of the fetus were observed at the Columbia Hospital and the Milwaukee Children's Hospital. Three of the 5 cases, although not associated with industry, could be attributed to handling of painted toys and lead soldiers. The fourth case was that of a man of 63 years with severe anemia and leukopenia. He had never been associated with an occupation which exposed him to a hazard of lead poisoning. After all other leads toward an explanation of the anemia had failed, lead was considered as a possible cause of his illness. He was found to excrete appreciable amounts of lead in the urine, and the lead content of the water in his home was sufficient to establish a lead hazard. Following an injury of the finger, staphylococcic septicemia developed, and the patient died of the infection. At postmortem examination all organs contained marked amounts of lead. The fifth case was that of a premature infant, one of twins, who was found to have a marked amount of lead in the ribs and an unusual amount of lead in the liver. The mother had had another child born prematurely, who remained underdeveloped. During a subsequent pregnancy the mother had increasing amounts of lead in the urine during gestation, and at the time of delivery the placenta was found to contain lead. These experiences with lead absorption and intoxication stimulated the present study.

We determined to establish the absorption of lead in the various age groups from early gestation throughout the life span of man in persons not exposed to known hazards of lead poisoning. We also wished to

From the Columbia Hospital and the Milwaukee Children's Hospital.

From the Laboratories of Pathology and Radiology, Columbia Hospital.

The record of the case reported was made available by Drs. R. E. Morter and C. W. Long.

This investigation was aided by a grant from the Howard E. Mitchell Research Fund.

know the distribution of lead in the various organs during the different types of illness. For this purpose we chose the rib and liver as organs to be routinely examined, because these organs were easily and immediately available on opening the body during postmortem examination. Other reasons for choosing these organs were that they contained appreciable amounts of lead in known absorptions, and the rib would be the most accessible bone should we ever care to do a biopsy of the skeleton.

PRESENTATION OF DATA

The method of Fairhall, used in these determinations, was adopted by the Industrial Commission of Wisconsin years ago for the determination of compensation in cases of injury alleged to be due to lead poisoning. During this time more than 1,000 examinations were made by Perry for evaluating legal and workman's compensation claims, as well

TABLE 1.—*Lead Content of Entire Fetuses*

Age, Weeks	Obstetric Condition	Dried Weight, Gm.	Lead, Mg. per 100 Gm.	Mg. per 100 Gm.
12	Uterine bleeding.....	4.2951	1.105	25.7
11	Uterine bleeding; twin pregnancy.....	0.3250	0.028	8.6
14	Uterine bleeding.....	3.7848	0.034	0.9
16	Uterine bleeding.....	8.0538	0.002	0.7
24	Uterine bleeding.....	92.0808	Trace	0.0
10	Uterine bleeding; twin pregnancy.....	8.3480	0.000	0.0
16	Toxemia of pregnancy.....	11.8925	0.000	0.0
13	Uterine hemorrhage.....	2.6875	0.000	0.0

as for the purpose of controlling lead hazards for various industrial firms in and about Milwaukee. These determinations compared favorably with results obtained in a survey made by the United States Public Health Service with the dithizone method in one of the industrial plants in which we had made a great many determinations. We had, therefore, come to rely firmly on this method both because of its sound chemical principle and because of the results obtained with it.

The results of our studies on lead absorption and intoxication in fetuses and persons who had not been exposed to known hazards of lead poisoning are summarized in tables 1 to 5. All subjects had always, or for a long period of time, been residents of Milwaukee. The results should present accurately the absorption of lead in this locality.

Lead Content of Entire Fetuses (Table 1).—In accordance with the work of Baumann¹ and others, it appears that lead passes through the placenta into the fetal circulation and is deposited in the organs of the fetus in much the same way as lead absorbed after birth. The greatest concentration of lead in the tissues of our series occurred in the 2 youngest fetuses. This fact, when coupled with the fact that the concentrations

1. Baumann, A.: Arch. f. Gynäk. **153**:584, 1933.

in the other fetuses were relatively low, is indicative that the abortions of these 2 fetuses were due to lead intoxication. In our limited series this was an incidence of 25 per cent. Lead has long been known as a cause of abortion in lead workers.² The likelihood that it plays such a

TABLE 2.—*Lead in Fetal Ribs and Liver*

Age, Mo.	Obstetric Condition	Ribs, Mg. per 100 Gm.*	Liver, Mg. per 100 Gm.*
6	Lead intoxication (?).....	23.058	4.650
7	Not known.....	6.988	2.009
7½	Nephritic toxemia.....	5.841	1.937
7	Pregnancy toxemia.....	5.577	2.417
4½	Pregnancy toxemia.....	5.524	0.807
9	Hydronephrosis (fetus); intrauterine septicemia.....	4.506	2.523
9	Preeclampsic toxemia.....	2.410	2.050
6	Asphyxia pallida (fetus).....	1.416	0.000
9	Erythroblastosis (fetus).....	1.042	0.909
6	Intestinal influenza.....	0.000	0.000
7	Placenta praevia.....	0.000	0.000

* Dried weight.

TABLE 3.—*Lead Content of Liver and Ribs from Birth to Ninety-Three Years of Age*

Age	Disease	Ribs, Mg. per 100 Gm.*	Liver, Mg. per 100 Gm.*
55 yr.	Carcinoma.....	8.642	0.796
5 mo.	Pneumonia.....	8.345	3.103
93 yr.	Aneurysm.....	7.904	2.289
65 yr.	Lead intoxication, septicemia.....	5.905	21.033
1 day	Premature infant (6 mo.).....	4.847	1.515
2 days	Congenital pneumonia.....	4.830	2.288
40 yr.	Cerebral hemorrhage, nephritis.....	4.749	4.374
60 yr.	Carcinoma.....	3.101	1.740
44 yr.	Pericarditis.....	3.007	1.431
2 yr.	Pneumonia.....	2.829	1.324
17 yr.	Sulfide.....	2.735	2.399
2 mo.	Hydronephrosis.....	2.321	0.965
3 yr.	Diabetes.....	2.314	2.713
68 yr.	Abscess of liver.....	2.046	2.688
7 mo.	Hydrocephalus.....	1.886	1.800
4 days	Erythroblastosis.....	1.836	0.374
50 yr.	Tuberculous pneumonia.....	1.637	1.700
62 yr.	Pneumonia, nephritis, diabetes.....	1.464	1.022
33 yr.	Malignant lymphoma.....	1.378	1.469
29 yr.	Empyema.....	1.202
2 days	Congenital pneumonia.....	1.119	1.633
4 yr.	Septicemia.....	0.802	1.082
9 yr.	Pneumonia.....	0.503	0.927
3 yr.	Glioma of brain.....	0.120	1.369
3.5 mo.	Meningitis.....	0.000	0.603
11 mo.	Pneumonia, empyema.....	0.000	1.325
5 yr.	Perforated appendix.....	0.000	0.000
12 yr.	Peritonitis.....	0.000	0.000
7 mo.	Whooping cough, pneumonia.....	0.000	0.000

* Dried weight.

role in workers other than lead workers is not appreciated. More determinations of lead in fetuses under 12 weeks of gestation appear indicated.

Lead in Fetal Ribs and Liver (Table 2).—The concentration of lead in the ribs of each fetus was greater than that in the liver. Only one

2. Oliver, T.: Brit. M. J. 1:1096, 1911.

stood out above all others in amount of lead absorbed. We have reason to believe that the mother suffered somewhat from lead intoxication, as indicated in table 5. We believe that lead intoxication was responsible for this premature birth. Even though 5 other fetuses listed in this table absorbed a considerable amount of lead, it appears to have been deposited for the most part in the skeleton, where lead is thought to be innocuous.

Lead in Ribs and Liver from Birth to the Age of 93 Years (Table 3).

—The fourth subject had well defined lead poisoning, as indicated in table 5. It is interesting to note that 3 other patients had distinctly more lead in the rib than this adult but markedly less in the liver. If a situation had risen whereby the lead of these persons had been mobilized from the skeleton, as in senile atrophy of the skeleton, a condition in

TABLE 4.—*Lead Content More in Liver Than in Rib Disease*

Age	Disease	Temperature, F.	Liver, Mg. per 100 Gm.	Rib, Mg. per 100 Gm.
63 yr.	Lead poisoning, septicemia.....	105	21.033	5.905
68 yr.	Abscess of liver.....	100	2.688	2.046
3 yr.	Diabetes, acidosis.....	104	2.713	2.314
50 yr.	Tuberculous pneumonia.....	100	2.688	2.046
62 yr.	Diabetes, nephritis, pneumonia.....	101	1.922	1.464
33 yr.	Malignant lymphoma.....	100	1.469	1.378
4 yr.	Septicemia.....	107	1.082	0.802
9 yr.	Pneumonia.....	106	0.927	0.508
3 yr.	Glioma.....	105	1.369	0.120
3 days	Congenital pneumonia.....	101	1.633	1.119
3.5 mo.	Meningitis.....	106	0.603	0.000
11 mo.	Pneumonia, empyema.....	105	1.325	0.000

which elderly persons lose upward of 50 per cent of their skeletal calcium, these patients might also have suffered from lead intoxication. The hazard was present, but apparently such a hazard rarely asserts itself. Absorption of lead appears to be one thing and intoxication from lead another. Lead absorption is a chemical determination, and lead intoxication is a clinical condition in which absorption of lead is but a factor.

The sources of the lead normally absorbed were well pointed out by Kehoe.³ Soil, food and water contained lead in varying amounts even in remote, almost isolated Mexican localities. The absorption of lead in these isolated areas was not as great as in the industrialized areas about Cincinnati. This distinction between town and country absorption apparently does not hold for more thickly populated Europe.

Lead Content of Liver Greater Than That of Rib in Disease (Table 4).—The first patient listed in table 4 was the only one suspected of having lead poisoning. The high concentration of lead stands out prominently above all others. The table reveals that not infrequently in illness

3. Kehoe, R. A.; Thamann, F., and Cholak, J.: J. Indust. Hyg. 15:290, 1933.

the soft tissue has a higher concentration of lead than the skeleton. This observation is in accordance with the experience of Fox and Ramage⁴ and others. Alert clinical observation and determinations of the lead excreted are necessary in order to establish properly the importance of lead as a factor in various diseases.

Probable Lead Intoxication (Table 5).—In 4 of the 48 subjects lead intoxication was indicated. Since 3 of the subjects were fetuses the intoxication was associated with pregnancy, and in 2 instances the pregnancy was of twelve weeks' duration or less. These 2 cases were thought

TABLE 5.—*Probable Lead Intoxication*

Age	History	Ribs, Mg. per 100 Gm.*	Liver, Mg. per 100 Gm.*
Fetus, 6 mo.	A premature underdeveloped child. Urinary lead excretion 0.125 mg. in 24 hr. Lead excretion in urine of mother, again pregnant, 0.114, 0.144, 0.125, 0.3 and 0.552 mg. in 24 hr. Placental tissue yielded 1.959 mg. per 100 Gm.	23.058	4.65
Adult, 65 yr.	Hypogenic hemopoiesis, eosinophils, polychromasia, stippled red cells. Water lead 0.08 mg. per liter; urinary lead 0.390, 0.420, 0.270 and 0.091 mg. in 24 hr.	5,905	21,033
Fetus, 12 weeks	Uterine hemorrhage	Entire Fetus, Mg. per 100 Gm.*	
Fetus, 12 weeks	Uterine hemorrhage	25.7	8.6

* Dried weight.

TABLE 6.—*Factors for Converting Fresh Weight to Dry Weight and Fresh Weight to Ash*

Tissue	Percentage Water	Factor	Percentage Ash	Factor
Teeth.....	10	1.11	72.5	1.38
Brain.....	85	6.66	1.5	66.60
Soft tissue.....	80	5.00	2.0	50.00
Bone.....	66	3.00	20.0	5.00
Cartilage.....	60	2.50	0.5	200.00
Entire fetus.....	80	5.00	2.0	50.00

to be instances of lead poisoning of the fetus because of the high concentration of lead in the tissue. The question naturally arises as to the vulnerability of the fetus to lead intoxication before a well defined skeleton has developed. Does calcification of the skeleton withdraw lead from the soft tissue and thus protect the fetus? The third subject was a 6 month fetus, one of twins, who lived several hours after birth. Both fetuses were born with peculiar lividity of the extremities. The history of the mother revealed that another child had been born prematurely. The pediatrician reported that this child had never developed normally either physically or mentally. Since lead is reported as a cause for

4. Fox, H. M., and Ramage, H.: Proc. Roy. Soc., London, s.B 108:157, 1931.

such underdevelopment,¹ a determination of lead was made on the child's urine. The content of lead in a twenty-four hour specimen was 0.125 mg. The mother again became pregnant. Lead determinations were made on the urine of the mother during the course of this gestation. The values were 0.114, 0.144, 0.125, 0.3, and 0.552 mg. per twenty-four hour specimen. The placenta contained 1.959 mg. of lead per hundred grams of dried weight. No stippled red blood cells were noted.

The fourth subject was a 65 year old man. He was referred to the laboratory for corroboration of a clinical diagnosis of pernicious anemia. Free acid in the stomach contents was demonstrated. There was no response to the various preparations which stimulate erythropoiesis. In fact, the production of formed elements from all bone marrow cells was distinctly depressed. There was moderate elevation in eosinophils, and stippled red cells were seen occasionally. The case resembled very closely a case of lead poisoning reported by Byfield.⁵ The literature indicates that rather acute lead poisoning leads to stimulation of bone marrow, while chronic poisoning leads to hypoactivity of bone marrow. Lead oxide and lead acetate have been employed to produce abortion. Anemia resembling pernicious anemia⁶ in the mother and an anemia resembling the so-called von Jacksch⁷ and Luzet type of anemia in the fetus are reported. The blood smear in these instances contained immature bone marrow cells. Megaloblasts and megalocytes were not present. From this information it appears that the initial effect of lead is that of an irritant to the bone marrow, while the later effect is that of a long-continued mild intoxication with the bone marrow hypogenic in consequence. The case under consideration falls into the latter category and resembles the cases of long-continued absorption as seen in printers and painters. One of us (G. H. H.) has, on several occasions, seen in elderly persons an anemia resembling pernicious anemia, which could not be classified. Elderly persons often lose more than 50 per cent of skeletal calcium as indicated by roentgen pictures. During this mobilization of calcium the lead of the skeleton is also mobilized. Studies of the excretion of lead are indicated in bizarre anemias of elderly persons.

REVIEW OF THE LITERATURE

A review of the literature and a summary of the results reported in this paper are presented in tables 7 and 8. Results of analyses in this laboratory which have not been published are included. The review, though, excepted, was limited to organs not in contact with the external world. These analyses were reported in three ways: results for fresh weight, results for dried weight and results for ash. The results for a

5. Byfield, A. F.: *Illinois M. J.* **33**:104, 1918.

6. Husfeldt, E.: *Acta obst. et gynec. Scandinav.* **8**:25, 1929.

7. Auban, P.: *Arch. de méd. d. enf.* **26**:297, 1923.

TABLE 7.—Lead Determinations on Human Subjects

Author	Subjects	Fresh Weight			Dry Weight			Ash		
		Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean
Teeth										
Bagchi, K. N.; Ganguly, H. D., and Sirdor, J. M.: Indian J. M. Research 24: 935, 1929.	4	0.04	0.003	0.034*	0.20	0.015	0.170	2.0	0.15	1.70
Pfrien, F.: Arch. f. Hyg. 111: 232, 1934.	54	6.217	0.435	3.232	6.901	5.383	3.587	8.58	0.67	4.46*
Lynch, G. R.; Slater, R. H., and Oaker, T. G.: Analyst 59: 787, 1934.	5	24.75	4.25	10.64*	27.472	4.717	11.81	34.155	5.805	14.583
Maulbetsch, A., and Rutishauser, E.: Arch. internat. de pharmacodyn. et de therap. 53: 55, 1936.	31	5.652	1.014	3.674	6.274	1.126	4.078	7.8	1.4	5.07*
Bagchi et al. (reference given above).	6	2.3	1.55	2.07*	2.553	1.72	2.297	3.174	2.139	2.856
Average mean			3.688			4.093			5.079	
Humerus										
Bagchi et al. (reference given above).	1	3.9	3.9*	11.7	11.7	19.5	19.5
Lynch et al. (reference given above).	3	5.5	4.1	4.43	16.5	12.3	13.29	27.5	20.5	22.15
Average mean			4.297			12.891			21.485	
Maxilla										
Maulbetsch and Rutishauser (reference given above).	10	1.22	0.44	0.798	3.06	1.32	2.394	6.1	2.2	3.99*
Average mean			0.798			2.394			3.99	
Calvarium										
Maulbetsch and Rutishauser (reference given above).	10	0.44	0.08	0.264	1.32	0.24	0.792	2.2	0.4	1.32*
Average mean			0.264			0.792			1.32	
Skull										
Bagchi et al. (reference given above).	1	1.43	1.43*	4.44	4.44	7.4	7.4
Average mean			1.43			4.44			7.4	
Pancreas										
Kehoe, R. A.; Thamann, F., and Cholak, J.: J. Indust. Hyg. 15: 273, 1933.	2	0.04	Trace	0.02*	0.20	0.10	2.0	1.0
Hansmann, G. H., and Perry, M. C.: Arch. Path., this article	1	3.396	3.396	16.981	16.981*	169.81	169.81
Average mean			1.145			5.725			57.25	
Brain										
Weyrauch, F., and Muller, H.: Ztschr. f. Hyg. u. Infektionskr. 115: 216, 1933.	6	0.030	0.02	0.024*	0.1908	0.1382	0.1508	1.908	1.322	1.508
Kehoe et al. (reference given above).	1	0.003	0.003*	0.0533	0.0533	0.533	0.533
Hijman, A. J.: Far East A. Trop. Med., Tr. Ninth Cong. 1: 373, 1934.	13	0.36	0.0	0.126	2.4	0.00	0.34*	24.0	0.8	8.4
Bagchi et al. (reference given above).	5	0.010	0.00	0.0073*	0.0066	0.0	0.0450	0.666	0.0	0.466
Average mean			0.073			0.456			4.86	

TABLE 7.—Lead Determinations on Human Subjects—Continued

Author	Subjects	Fresh Weight			Dry Weight			Ash		
		Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean
Bagchi et al. (reference given above).....	5	0.047	0.005	0.028*	0.235	0.025	0.140	2.35	0.25	1.40
	Average mean		0.028			0.140			1.40	
Kehoe et al. (reference given above).....	6	0	0	0	0	0	0	0	0	0
	Ovary									
Kehoe et al. (reference given above).....	2	Trace*	0.045	0.056*	0.375	0.225	0.280	3.75	2.25	2.80
	Heart									
Bagchi et al. (reference given above).....	5	0.075	3.454	17.271	17.271*	172.71	172.71
	Hausmann and Perry (this article).....	1	3.454	2.324	23.24
	Average mean		0.467							
Kehoe et al. (reference given above).....	1	0	0	0*	0	0	0	0	0	0
	Prostate									
Kehoe et al. (reference given above).....	1	0	0	0*	0	0	0	0	0	0
	Bladder									
Tompsett and Anderson (reference given above).....	20	1.47	0.2	0.71*	4.41	0.6	2.13	7.35	1.0	3.55
	Tompsett, S. L.; Blochem, J. 30:345, 1936.....	19	1.65	0.46	0.83*	4.95	1.38	2.40	8.25	4.15
	Maulhetch and Rutishauser (reference given above).....	14	0.92	0.38	0.582	2.76	0.84	1.746	4.6	1.4
Average mean			0.719		2.157			3.595		
Tompsett and Anderson (reference given above).....	9	0.088	0.026	0.05*	0.440	0.130	0.250	4.40	1.30	2.50
	Kehoe et al. (reference given above).....	2	0.032	0.00	0.16*	0.0	0.80	0.160	0.0	8.0
	Bagchi et al. (reference given above).....	5	0.06	0.03	0.045*	0.30	0.15	0.225	3.0	1.5
Average mean			0.062		0.311			3.11		
Tompsett, S. L. (reference given above).....	10	10.83	1.82	5.0*	32.40	5.46	15.0	54.15	9.10	25.00
	Lynch et al. (reference given above).....	25	13.25	2.35	5.96*	39.75	7.06	17.97	66.25	11.76
	Maulhetch and Rutishauser (reference given above).....	18	0.66	0.07	0.303	1.98	0.21	0.906	3.3	0.35
Bagchi et al. (reference given above).....	2	2.26	1.2	1.73*	6.78	3.0	5.19	11.30	6.0	
Hausmann and Perry (this article).....	1	2.583	2.583	7.749	7.749*	12.915	12.915
Average mean			2.942		11.898			19.71		

Bagchi et al. (reference given above).....	2	1.45	0.68	1.01*	4.85	2.04	3.08	7.25	2.40	5.05
Tompsett, S. L. (reference given above).....	19	0.63	1.53	4.25*	32.95	4.59	12.75	48.35	7.85	21.25
Average mean			3.941			11.324			19.66	
Bagchi et al. (reference given above).....	3	0	0	0*	0	0	0	0	0	0
Kehoe et al. (reference given above).....	2	0	0	0*	0	0	0	0	0	0
Fat										
Hijman, A. J. (reference given above).....	3	17.83	0.5	6.466	53.5	1.5	19.4*	89.15	2.5	32.33
Weyrauch and Muller (reference given above).....	36	1.8	0.333	0.8	5.4	0.599	2.4	9.0	1.66	4.0*
Barth, E.: Virchows Arch. f. path. Anat. 281 : 146, 1931.....	30	0.5203	0.1644	0.342	1.5003	0.4632	1.026	2.601	0.822	1.711
Average mean			0.847			2.541			4.235	
Bagchi et al. (reference given above).....	4	0.04	0.003	0.034*	0.20	0.015	0.170	2.0	0.15	1.70
Average mean			0.034			0.170			1.70	
Testes										
Tompsett and Anderson (reference given above).....	20	0.463	0.085	0.173*	2.315	0.425	0.865	23.15	4.25	8.65
Kehoe et al. (reference given above).....	2	0.08	0.051	0.079*	0.40	0.255	0.395	4.0	2.55	3.95
Lynch et al. (reference given above).....	5	0.18	0.00	0.136*	0.90	0.00	0.690	9.00	0.00	6.90
Weyrauch and Muller (reference given above).....	40	0.24	0.02	0.0344*	1.20	0.10	0.172	12.00	1.00	1.72
Hijman, A. J. (reference given above).....	7	3.06	0.00	0.697*	15.3	0.00	3.485*	153.00	0.00	34.85
Bagchi et al. (reference given above).....	9	0.062	0.031	0.057*	0.410	0.155	0.285	4.10	1.55	2.85
Hansmann and Perry (this article).....	28	4.206	0.00	0.372	21.033	0.00	1.859*	210.33	0.00	18.99
Average mean			0.193			0.905			9.65	
Tompsett and Anderson (reference given above).....	20	1.29	0.157	0.835*	3.87	0.471	2.565	6.45	0.785	4.275
Kehoe et al. (reference given above).....	19	1.75	0.4	0.997*	3.25	1.2	2.991	8.85	2.0	4.965
Minot and Aub (reference given above).....	2	0	0	0*	0	0	0	0	0	0
Maulhatsch and Rutishauser (reference given above).....	15	1.16	0.20	0.496	3.48	0.6	1.488	5.8	1.0	2.48*
Bagchi et al. (reference given above).....	2	0.85	0.00	0.835*	2.56	2.46	2.906	4.25	4.1	4.175
Hansmann and Perry (this article).....	29	2.881	0.0	0.57	8.642	0.0	2.61*	14.405	0.0	4.35
Average mean			0.811			2.433			4.056	
Tompsett and Anderson (reference given above).....	19	0.355	0.072	0.185*	1.775	0.360	0.675	17.75	3.60	6.75
Kehoe et al. (reference given above).....	2	0.07	0.059	0.084*	0.35	0.236	0.359	9.5	2.36	3.59
Kehoe, Thannann and Cholak *.....	46	0.04	0.02	0.0244*	0.20	0.10	0.123	2.0	1.0	1.22
Hijman, A. J. (reference given above).....	5	2.19	0.06	0.688*	15.6	0.00	3.48	156.0	0.0	34.8
Bagchi et al. (reference given above).....	8	0.071	0.037	0.068*	0.355	0.185	0.280	3.55	1.85	2.90
Hansmann and Perry (this article).....	1	2.211	2.211	11.055	11.055*	110.55	110.55
Average mean			0.189			0.651			6.51	
Kidney										

* The state in which the examination was made is indicated by an asterisk. The results for a state other than that in which the examination was made were computed.

TABLE 8.—Lead Determinations on Fetuses

Author	Fetuses	Fresh Weight			Dry Weight			Ash		
		Maximum		Mean	Maximum		Mean	Maximum		Mean
		Minimum	Minimum	Minimum	Minimum	Minimum	Minimum	Minimum	Minimum	
Liver										
Tompsett, S. L., and Anderson, A. B.: <i>Biochem. J.</i> 29: 1851, 1935	4	0.095	0.033	0.068*	0.475	0.165	0.340	4.75	1.65	3.40
Baumann, A. ¹	4	0.18	0.016	0.060*	0.90	0.080	0.330	9.0	0.80	3.30
Hanemann and Perry (this article).....	11	0.920	0.0	0.316	4.650	0.0	1.583*	46.5	0.0	15.83
Average mean			0.211			1.057			10.37	
Rib										
Hanemann and Perry (this article).....	11	7.686	0.0	1.708	23.053	0.0	5.125*	38.43	0.0	8.54
Average mean			1.708			5.125			5.34	
Kidney										
Tompsett and Anderson (reference given above).....	4	0.067	0.003	0.065*	0.335	0.315	0.325	3.35	3.15	3.25
Bagchi, K. N.; Ganguly, H. D., and Sirdor, J. M.: <i>Indian J. M. Research</i> 26: 955, 1939.....	4	0.033	0.006	0.018*	0.165	0.030	0.09	1.65	0.3	0.9
Average mean			0.0415			0.2075			2.075	
Brain										
Tompsett and Anderson (reference given above).....	4	0.067	0.003	0.0045*	0.406	0.0193	0.0299	0.466	0.198	0.299
Bagchi et al. (reference given above).....	3	0.016	0.00	0.0036*	0.1066	0.00	0.0373	1.066	0.00	0.373
Hilman, A. J.; <i>Far East A. Trop. Med., Tr. Ninth Cong.</i> 1: 373, 1934	13	0.306	0.00	0.012	2.44	0.0	0.084*	24.4	0.0	0.84
Average mean			0.00054			0.06617			0.0617	
Femur										
Tompsett and Anderson (reference given above).....	4	0.906	0.130	0.175*	0.793	0.390	0.525	1.33	0.63	0.875
Average mean			0.175			0.525			0.875	

Bagchi et al. (reference given above).....	4	Average mean	Lung	0.02	0.0	0.005	0.005*	0.10	0.0	0.025	1.0	0.0	0.25
Bagchi et al. (reference given above).....	2		Thymus	0.00	0.00	0.00	0.00*	0.00	0.00	0.10	0.00	0.00	0.10
Bagchi et al. (reference given above).....	2	Average mean	Spleen	0.005	0.00	0.0025	0.0025*	0.025	0.0	0.0125	0.25	0.0	0.125
Bagchi et al. (reference given above).....	1	Average mean	Sternum	0.04	0.04	0.04*	0.12	0.12	0.20	0.20
Bagchi et al. (reference given above).....	2	Average mean	Long Bone	0.6	0.4	0.5	0.5*	1.8	1.2	1.5	3.0	2.0	2.5
Bagchi et al. (reference given above).....	1	Average mean	Flat Bone	0.325	0.325	0.325*	0.975	0.075	1.025	1.025
Bagchi et al. (reference given above).....	1	Average mean	Bladder	0.005	0.005	0.005*	0.025	0.025	0.25	0.25
Hansmann and Perry (this article).....	10	Average mean	Entire Fetus	5.14	0.0	0.718	0.718	25.7	0.0	3.59*	257.0	0.0	35.9

* The state in which the examination was made is indicated by an asterisk. The results for a state other than that in which the examination was made were computed.

state other than that in which the examination was made were computed. The state in which the examination was made is indicated by an asterisk. The factors used in computing values are given in table 6. The tabulation of actual determinations integrated with computed values is offered as a more comprehensible view of the status quo of lead absorption when no history of unusual absorption of lead is involved.

SUMMARY

The analysis of the data obtained may be divided into facts derived from the data and suggestions for consideration contained in the data.

A. Facts derived from the data:

1. Examinations of tissues for lead absorption were made on 48 bodies, the ages of which ranged from 11 weeks' gestation to 93 years of age.
2. The amount of lead in the ribs varied from 23.058 mg. to 0.00 mg. per hundred grams.
3. The amount of lead in the liver varied from 21.033 mg. to 0.00 mg. per hundred grams.
4. The lead content is expressed in milligrams per hundred grams of dried tissue.
5. There appeared to be no relationship between the amount of lead absorbed and the age except that those subjects containing no lead were either fetuses or children under 12 years of age.
6. The amount of lead in the liver may exceed that in the rib during prolonged metabolic disturbances, severe infections and less acute progressive fatal illness.
7. A mother who has absorbed lead will excrete increasing amounts of lead during gestation, the excretion of which parallels the skeletal growth of the fetus.
8. The analysis of entire fetuses, the ages ranging from 11 to 24 weeks' gestation, revealed lead in 62.5 per cent of them. The amount in 25 per cent of these bodies may be considered hazardous.
9. Of fetuses from 4½ months to term, 80 per cent had lead in the rib or the liver or in both organs.
10. All subjects over 12 years of age or 90 per cent of those between birth and 93 years of age revealed evidence of lead absorption.
11. A review of the results recorded in the literature, with which our results were integrated, has established by spectrographic, colorimetric and titrametric methods that lead absorption is a normal consideration.
12. The cases were all drawn from Milwaukee and its vicinity.

B. Suggestions for consideration contained in the data:

1. Lead absorption may act as an unrecognized factor in various diseases in persons who cannot trace their exposure to lead.
2. Aged persons, who frequently lose, often quite rapidly, upward of 50 per cent of their skeletal calcium, may suffer symptoms of intoxication from the lead which is concurrently released, provided the skeleton is heavily loaded.
3. Women may suffer from lead intoxication during gestation, owing to the fact that much lead is released from a heavily loaded skeleton during gestation. This may be responsible for some of the anemias closely resembling pernicious anemia which occur during and shortly after pregnancy.
4. The fetus is likewise exposed to lead hazards, which may result in intoxication of the fetus or expulsion of the fetus as a result of the action of lead on the uterus.
5. The patient with a heavily loaded skeleton may suffer from symptoms of lead intoxication during uncontrolled metabolic diseases, severe infections or prolonged, progressive illness owing to the fact that lead is mobilized from the skeleton and fixed by the organs.
6. Lead may be an important cause of abortion during the first three months of gestation.
7. The development of the fetal skeleton may protect the fetus by withdrawal of lead from the circulation.
8. Lead absorption may become an individual problem and the concern of every physician.

AMYLOID

I. METHODS OF ISOLATING AMYLOID FROM OTHER TISSUE ELEMENTS

GEORGE HASS, M.D.*

AND

R. Z. SCHULZ, M.D.

NEW YORK

"Amyloid" is a term which is used to designate a product that appears as a formed element in certain intercellular regions during the course of seemingly diverse pathologic states. It is characterized morphologically by its deposition in elective sites, its more or less dependable affinities for several unrelated stains and its optical homogeneity.

The factors which govern the formation, deposition and removal of amyloid are unknown. Although it is generally regarded as a compound of protein with chondroitin-sulfuric acid, neither a purified protein nor chondroitin-sulfuric acid has ever been isolated from amyloid by acceptable chemical methods.

This report is concerned, first, with methods devised for separating amyloid from normal tissue elements; second, with a description of the proteins which were obtained by applying the methods, and, third, with a discussion of a hypothesis which may be of use in directing the study of amyloid disease.

MATERIAL

The material available for this study was obtained post mortem from patients who had chronic tuberculosis of the lungs. In the average case six to eight hours elapsed between the death of the patient and the beginning of our investigations. We restricted our studies to fresh livers, spleens and kidneys which contained a large amount of amyloid. Various steps in the methods were developed progressively by using eight livers and four spleens. The final methods were applied to five livers in succession with fairly consistent results.

METHODS

The first problem with which we were confronted was the development of a method for isolating amyloid from other microscopic tissue structures, at least from a morphologic point of view. An approach to this problem required a study of the relative susceptibilities of amyloid and other tissue components to the action of numerous solvents. Two methods of analysis were used in this study. One method consisted of a microscopic inspection of the tissue at intervals during

* Past Member Society of Fellows, Harvard University.

From the Departments of Pathology of Cornell University Medical College and Harvard Medical School.

the period of action of a solvent. This inspection was facilitated by using several different stains. The second method consisted of fractionation of proteins appearing in a solvent during various extraction intervals. The correlation of simultaneous histologic and chemical data obtained by these methods served as the source of our principal conclusions. In order that this correlation might be fairly reliable it was necessary to devise a uniform method of preparing normal and amyloid-bearing tissues.

In our first few experiments organs were perfused with physiologic solution of sodium chloride and then cut into small pieces before the action of various solvents was investigated. This method of preparing tissues was not satisfactory. Perfusion was not uniformly effective in removing the blood from vascular channels. The irregular fragments of tissue were unsuitable for microscopic study. Finally, the variable dimensions of the tissue fragments interfered with the action of solvents on amyloid and other tissue components. This method was therefore soon dispensed with, and in all studies to be reported here fresh tissues cut into thin sections with a freezing microtome were used. Tissues prepared in this way were satisfactory for the purposes of our investigations. The sections could be quickly extracted with water, alcohol or ether. Large numbers of sections could be manipulated efficiently with minimal difficulties incidental to filtration or centrifugation. Equality in thickness of the sections insured fairly equal rates of removal of materials from all tissue exposed to the solvent. Semiquantitative comparative studies could be done by working with equal numbers of sections simultaneously in several different solvents. A final very important advantage was that the microscopic state of amyloid in a large mass of individual sections could be accurately determined by removing a few sections at random from the solvent, staining them with congo red for one or two minutes and studying the stained mounted sections under the microscope at once.

Therefore, all studies reported here were done on fresh tissue cut with a freezing microtome into sections which averaged 25 to 30 microns in thickness and 2 or 3 cm. in length and breadth. Several hundred grams of liver and smaller amounts of spleen and kidney were prepared routinely in this way after completion of the postmortem examination. The sections were then washed for two to three hours in aqueous 1 per cent sodium chloride solution, the solvent being changed frequently. When saturation of samples of the solvent with solid ammonium sulfate failed to produce a precipitate, the washing was terminated. Sections so prepared were available for studies, which were usually carried out promptly.

A number of experiments were done in order to establish conditions under which amyloid might be either removed from the tissues or altered in some significant way. Various solvents were employed, not only at neutrality but also in the acid and alkaline range. Observations on sections were made periodically, the majority of the experiments running for five to seven days before final conclusions were drawn. Brief experiments were conducted at room temperature and the remainder at 5 C.

Neutral aqueous salt solutions, namely, 1 per cent sodium chloride, 2 per cent sodium chloride, 1 per cent potassium iodide, 2 per cent potassium iodide and 10 per cent calcium chloride, were used as potential solvents.

Sections were boiled in distilled water under a reflux condenser at 100 C. for five days.

Sections were placed in the following acid solutions: first, hydrochloric acid in the following strengths—10 N, N, 0.1 N, 0.01 N, 0.001 N; second, hydrochloric acid-citrate buffer solutions ranging in steps of less than 1 p_H unit from p_H 1 to 5; third, acetate buffer solutions from p_H 4 to 5.6; fourth, phosphate buffer solutions from p_H 5.3 to 7.1, and fifth, glacial acetic acid.

Alkaline aqueous solutions were used with and without a small quantity of sodium chloride, as follows: calcium hydroxide ($\text{Ca}[\text{OH}]_2$), saturated and half-saturated; barium hydroxide ($\text{Ba}[\text{OH}]_2$), saturated and half-saturated; sodium hydroxide, 0.001 N, 0.002 N, 0.005 N, 0.01 N, 0.02 N, and 0.1 N; standard borate buffer solutions ranging from about p_H 8 to 12, and, finally, standard phosphate buffer solutions, p_H 8.04 to 12.06.

Sections which had been treated with alcohol, 10 per cent formaldehyde, 10 per cent calcium chloride and acetate buffer solutions were washed in aqueous 1 per cent sodium chloride solution and placed in the series of standard alkaline phosphate buffer solutions. Sections "deformaldehydized" at p_H 4 were treated in a similar way, together with appropriate controls.

In another series of experiments the sections were placed in alkaline buffer solutions containing about 1, 5 and 10 per cent sodium chloride.

Dehydrated and hydrated sections were placed in anhydrous ethanolamine, a strong organic base.

Birefringent properties of amyloid were sought for in four livers and two spleens* by use of a polarizing microscope. The amyloid of one liver possessed nonhomogeneous optical birefringence. The influence of dyes, buffer solutions and organic fat solvents on this property was studied.

Although the methods which have been described were applied principally in the study of amyloid in the liver, several amyloid-bearing spleens and kidneys were investigated in a similar way. One of the spleens was from a patient who had received congo red intravenously two months before death. The amyloid in this spleen was dull red. The properties of this amyloid were such that we were obliged to undertake a study of dye-amyloid complexes. The results of this study will be reported at some later time.

After determining the influence of certain reagents on amyloid and establishing efficient differential limits of solubility in alkaline phosphate buffer solutions, the following tentative procedure for the isolation of amyloid protein was developed and controlled by a study of normal livers.

Fresh liver was cut into sections 25 to 30 microns in thickness with a freezing microtome. The sections, 100 to 200 Gm. in weight, were washed thoroughly in distilled water containing 1 Gm. of sodium chloride in 100 cc. The washing was continued until no precipitate was obtained by saturating samples of the solvent with ammonium sulfate. At this point two procedures were followed. When the quantity of amyloid in the sections was small in proportion to the quantity of hepatic parenchyma, especially when the parenchymal cells showed fatty degeneration, the sections were placed in 95 per cent alcohol, absolute alcohol, purified petroleum benzine U. S. P. (petroleum ether), 95 per cent alcohol and aqueous 1 per cent sodium chloride solution in succession before proceeding with the extraction. This step was designed to remove the lipoids. When amyloid was present in large amounts and when there was little fatty degeneration of the

parenchymal cells, this step was omitted. The best results were not obtained when the alcohol-ether treatment was used, but when contaminating lipoids interfered with the properties of the proteins in solution that treatment was a required procedure. After the mass of sections had been washed free from alcohol, they were placed in 500 to 1,000 cc. of a standard phosphate buffer solution at p_H 9.8 to which 5 to 10 cc. of 0.1 molar potassium iodate had been added. After the sections had stood in this solution at 5 C. for two hours, they were transferred to a second similar solution and the extraction repeated. The sections were then washed in 1 per cent sodium chloride solution and transferred to 1,000 cc. of a standard phosphate buffer solution at p_H 11 to which 5 to 10 cc. of 0.1 molar potassium iodate had been added. The solution rapidly became opalescent when the sections contained amyloid. Periodic swirling of the sections and replenishment of the buffer solution resulted in fairly complete removal of the amyloid from the tissues within four to six hours. For complete removal a somewhat longer period of time, which seemed to depend chiefly on the quantity of buffer solution, was required. Our most rapid gross extractions were completed in eight hours. Less time was required with microextractions.

The progress of the extraction was followed conveniently by periodically removing random sections, staining them with congo red, and making an immediate microscopic study of the sections mounted on glass slides. On completion of the extraction the sections were entirely free from amyloid. The solvent was opalescent, colorless and of relatively low viscosity.

Neutralization of the solution was accomplished either with carbon dioxide or with acetic acid. Carbon dioxide was preferable because of the danger of over-neutralization with acetic acid. For some purposes a fairly satisfactory preparation of a major component in the extraction fluid was obtained at this stage by adjusting the solution to p_H 4 to 5 with acetic acid. In this range almost all protein in the solution precipitated in white flocculent masses. Careful neutralization with acetic acid or carbon dioxide produced increased viscosity of the solution but no precipitate.

The neutralized solution was diluted with saturated aqueous ammonium sulfate, and solid ammonium sulfate was added to define the precipitation limits of the proteins. Inasmuch as half saturation was found to be adequate for precipitation of all protein, this was established as a standard. After standing at 5 C. over night, the protein was separated from the supernatant fluid by decantation and gentle centrifugation. The protein so obtained was redispersed in water, sufficient salt being present. A variable result was obtained at this point, namely, partial or complete solution of the protein. After the solution had stood for several hours, the insoluble components, when present, were filtered off and the solution dialyzed in cellophane sacs against running tap water for forty-eight hours. Dialysis was completed against distilled water in the refrigerator. The precipitate in the dialyzing sacs was thereafter either dispersed in dilute neutral salt solution and precipitated with acetic acid at p_H 4 to 5 or filtered on sintered glass prior to drying. Drying was accomplished with alcohol and purified petroleum benzine U. S. P. (petroleum ether) in the usual way.

The residual supernatant water-soluble protein was precipitated with alcohol and dried.

The dried materials were grayish white powders. One material represented the principal protein component of amyloid. The other substance, always present in small amounts, had different properties and may not be related to the amyloid complex.

Normal liver tissue in frozen sections was likewise subjected to this procedure. A comparison of the results with those obtained in the study of amyloid-bearing liver tissue yielded data which will be considered in the following paragraphs.

RESULTS

The amyloid of one liver appeared to possess the usual homogeneity by ordinary illumination, but birefringence was noted during studies with polarized light. The birefringence was nonhomogeneous, being distributed irregularly throughout the deposits. The property of double refraction was accentuated by treatment of the tissue with acids, compound solution of iodine U. S. P. (Lugol's solution) or congo red. It was unaffected by treatment with alcohol and ether. It disappeared during the immersion of sections in alkaline mediums at p_H 9.5 to 10 and did not reappear on subsequent acidification of the tissues. The cause of the birefringence was not determined. It was absent in sections of the amyloid spleen from the same patient.

Amyloid in sections was unchanged by exposure to aqueous solutions of 1 per cent or 2 per cent sodium chloride, 1 per cent or 2 per cent potassium iodide and 10 per cent calcium chloride.

Boiling water over a period of five days decreased the homogeneity of amyloid but did not dissolve the complex.

Amyloid was destroyed along with other tissue elements by prolonged treatment with 10 N hydrochloric acid. It was insoluble in N, 0.1 N, 0.01 N and 0.001 N hydrochloric acid. The acid in these concentrations, however, caused a general contraction in volume of amyloid in sections. The original volume was regained by exposure of the sections to slightly alkaline aqueous 1 per cent sodium chloride.

Except for visible contraction in volume, amyloid was indifferent to the action of glacial acetic acid, hydrochloric acid-citrate, acetate and phosphate buffer solutions covering the range p_H 1 to 7.1. The observed contraction in volume increased with increasing acidity of the medium between p_H 7.1 and p_H 5. At lower p_H values no further contraction was measurable by microscopic study.

Amyloid did not dissolve satisfactorily in saturated or half-saturated aqueous solutions of calcium hydroxide or barium hydroxide. A slight diminution in the quantity of amyloid was noted routinely in sections exposed to aqueous 1 per cent sodium chloride solution which was half saturated with barium hydroxide. Prolonged treatment of tissues with

barium hydroxide brought about such fragmentation of the sections that only granular remnants of amyloid and other tissue components were distinguishable on staining the tissue fragments with congo red. This method of treatment was of no use in studies concerned with isolating amyloid from other tissue elements.

Amyloid disappeared from fresh liver sections in 0.1 N, 0.02 N and 0.01 N aqueous solutions of sodium hydroxide. The disappearance was preceded by increasing optical homogeneity and swelling of the amyloid. When the quantity of solution was relatively great with respect to the amount of tissue, the amyloid was usually completely dissolved in the dilute alkaline solutions within six hours. An effective definable range of solubility lay between 0.02 and 0.01 N sodium hydroxide. Under these circumstances the cellular and interstitial components of the hepatic parenchyma remained normal in appearance.

By the use of alkaline borate and phosphate buffer solutions, more precise lower limits of solubility were defined. Appreciable amounts of amyloid began to disappear from the sections at p_H 10. The rate of disappearance was greatly increased at values approaching p_H 11 and was still more rapid at p_H 12. In general the phosphate buffer solutions at a given p_H were better solvent mediums than borate buffer solutions. In the standard phosphate buffer solution at p_H 10.97 amyloid began to disappear promptly from the sections and was completely removed within six hours in all test runs with sample sections. The variation in rapidity of solution among the several amyloid livers was appreciable but not of significance for our present purposes.

The brief alcohol-ether treatment of fresh frozen sections did not alter the lower limit of solubility as described. Neither was a previous treatment with 10 per cent calcium chloride (which is known to dissociate the chondroitin-sulfuric acid complexes of cartilage) effective in altering either the solubility or the staining properties of the amyloid in sections. Formaldehyde treatment of tissues, however, rapidly increased the resistance of amyloid to the action of the alkaline solvents, so that amyloid remained in the sections at p_H values well above those ordinarily satisfactory for its removal. "Deformaldehydization" of sections at p_H 4 over a period of several days did not restore the original solubility of the amyloid complex.

Staining with congo red also decreased the solubility of amyloid in the alkaline mediums. This effect was measurable when sections were stained very lightly. It increased in proportion to the depth of staining. That this was not only an *in vitro* effect was proved by an investigation of the amyloid spleen of a patient who had received congo red intra-

venously, as a test, two months before death. Solution of the amyloid-congo red complex of the spleen from this patient required a period in alkaline phosphate buffer medium three times that required for the average splenic amyloid. A complete study of dye-amyloid relationships will be discussed in another report. Suffice it to say that the conjunction of congo red with amyloid consistently altered the solubility of the natural compound under controlled in vitro circumstances. This combination was a very firm one, and congo red was released from amyloid, if at all, only under circumstances which led to the disappearance of amyloid from the sections.

The extraction of amyloid from the sections was satisfactory in phosphate buffer solution at p_H 11 which contained 1 per cent or 5 per cent sodium chloride though somewhat delayed in the solution in which the salt was more concentrated. Amyloid was insoluble, even at p_H 12, in a phosphate buffer solution containing 10 per cent sodium chloride. Although there was some precipitation of salts from this solution, and although a salt effect on p_H values should be considered in these experiments, the p_H as measured by alizarin yellow was well above that normally suitable for dissolving amyloid. This information was desirable because the amyloid proteins which were recovered from the extraction fluids were likewise insoluble in aqueous solutions of 10 per cent sodium chloride at a similar high p_H value.

Amyloid was insoluble in the strong organic base ethanolamine.

The methods for the removal of amyloid from the tissues were based on the solubility of amyloid in phosphate buffer solutions at p_H 10 to 11 and the retention of this property after rapid alcohol-ether extraction of lipoids from the sections. In the first place, it was desirable to remove as much nonamyloid tissue protein as possible prior to extraction of the amyloid. The preparation of thin frozen sections provided very satisfactory material for this purpose. All blood and other tissue proteins which are soluble in neutral salt solution were easily and rapidly removed from the tissues without the amyloid being changed in any recognizable way. The tissues were then subjected to alkaline extraction at a p_H just below that required for dissolving amyloid. In dealing with liver this procedure was satisfactory because all but a trace of normal liver proteins soluble in the range p_H 7 to 11 were removed in the range p_H 7 to 10. The subsequent extraction of amyloid at p_H 11 was therefore possible in the presence of a minimal contamination of the solvent with normal hepatic proteins (figs. 1 and 2).

Splenic amyloid possessed the same solubility properties as hepatic amyloid. However, in the range p_H 10 to 11 a large quantity of splenic

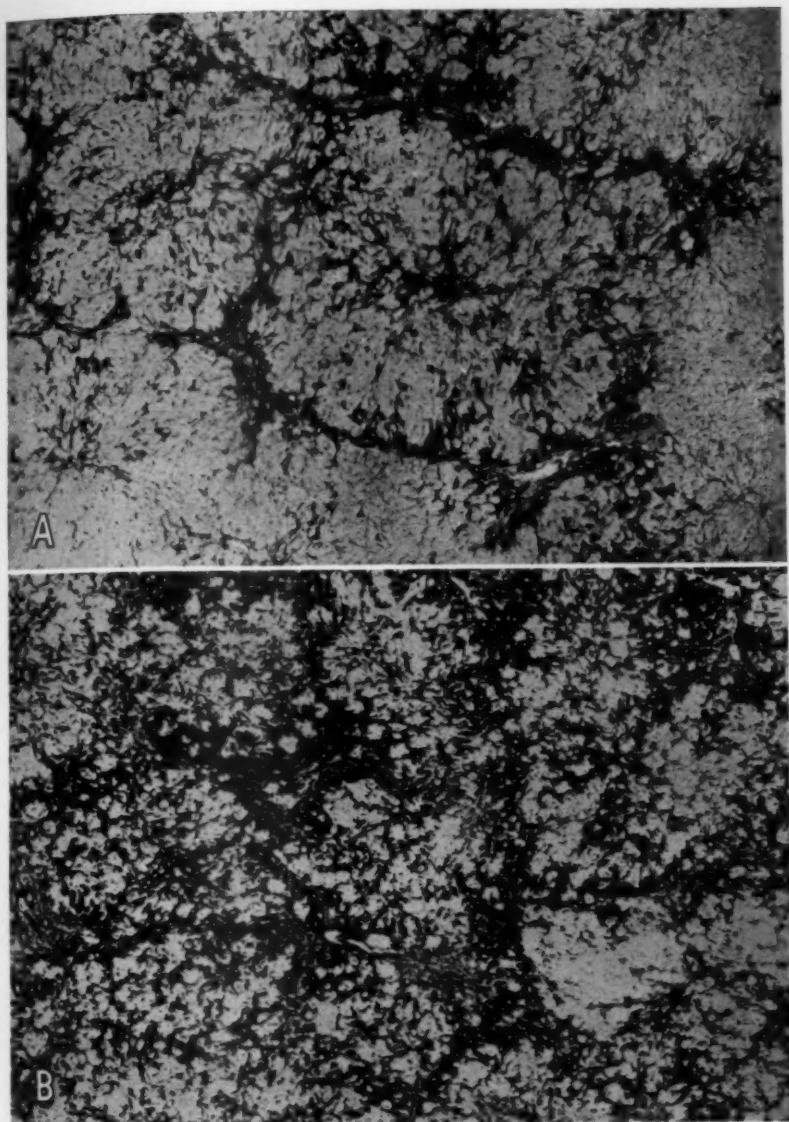


Fig. 1.—*A*, low power photomicrograph illustrating the appearance of amyloid in liver prior to the application of extraction methods. Zenker's fixation; phosphotungstic acid-hematoxylin stain. *B*, photomicrograph at the same magnification as *A*, showing the appearance of tissue from the same block of liver after extraction of the amyloid. Zenker's fixation; phosphotungstic acid-hematoxylin stain.

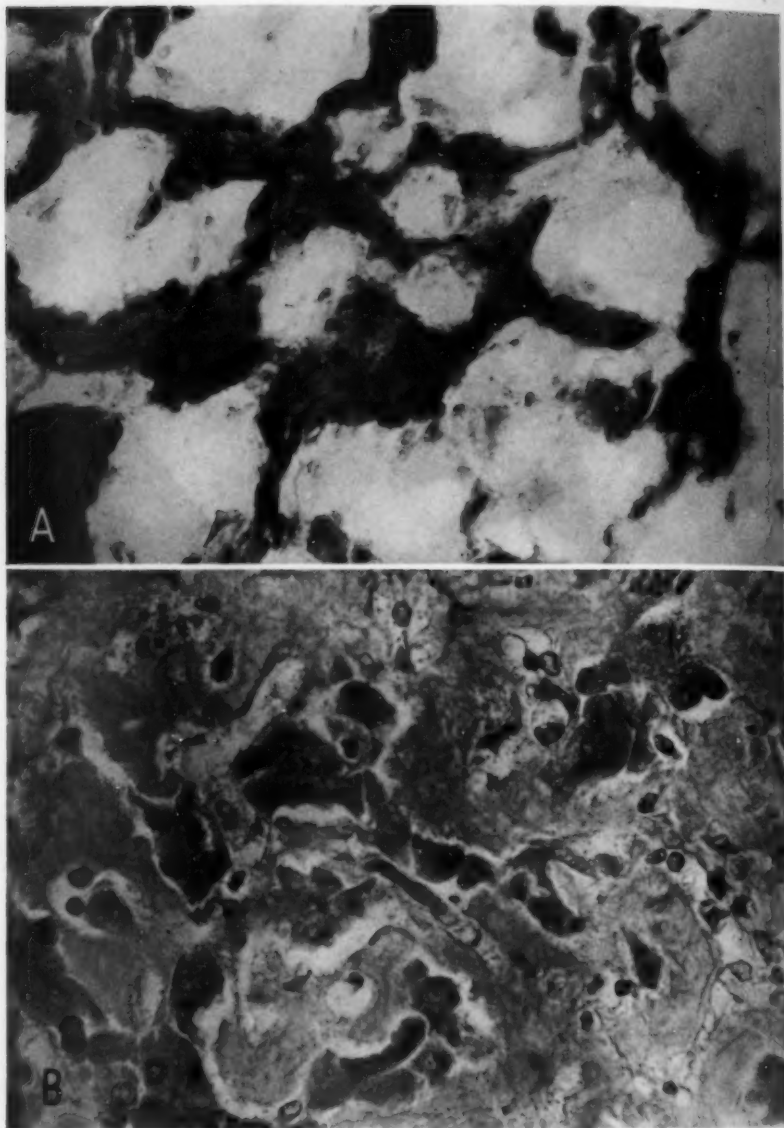


Fig. 2.—*A*, high power photomicrograph of a region of figure 1 *B*. Zenker's fixation; phosphotungstic acid-hematoxylin stain. *B*, high power photomicrograph of a region of figure 1 *A*. Note the removal of amyloid and the fair retention of cellular structure. Zenker's fixation; phosphotungstic acid-hematoxylin stain.

protein went into solution along with the amyloid (fig. 3). Most of this was probably splenic nucleoprotein, and inasmuch as its physical behavior was comparable to that of the principal amyloid fraction A, we concluded that the method was not satisfactory for the isolation of splenic amyloid.

Theoretically, the "sago" granules of amyloid in spleen should be suitable for mechanical methods of separation as employed by Hanssen.¹ A microscopic study of amyloid segregated by his method quickly dispelled the illusion produced by the gross particles. The average "sago" granule after mechanical isolation contained about as much enmeshed and adherent splenic structure as amyloid.

Renal amyloid was suitable for microscopic study of its properties (fig. 4). These were similar to those of hepatic and splenic amyloid. Because of the small quantity of amyloid in kidneys, the methods of gross extraction, as described, were inadequate.

The liver was well adapted for the application of the gross methods. The parenchyma and interstitial tissues of the liver were well preserved after extraction of the amyloid (fig. 1). The cords of liver cells remained intact. The collagenous and elastic tissues were not disturbed in a morphologic sense, although the sections tended to acquire increased extensibility. Endothelial cells, Kupffer cells and most nuclear and cytoplasmic structure remained morphologically intact, although it was clear that many compounds must have been removed from the cells during the extractions below p_H 10 (fig. 2).

The proteins which appeared in the extraction fluid at p_H 11 when sections of amyloid liver were under investigation remained in solution on neutralization of the solvent. If neutralization with acetic acid was carried too far, precipitation of protein began at p_H 6.4 and was maximal both as to rate and quantity at p_H 4 to 5.4. Invariably the precipitation of protein was incomplete. The protein persisting in the supernatant acidified solution was never present in an amount greater than 10 to 15 per cent of the total protein.

The protein precipitable with acetic acid may be designated as amyloid fraction A. The nonprecipitable protein may be designated as amyloid fraction B. The protein fraction A after precipitation with acetic acid could not be redispersed in solution at neutrality. It required the same alkaline phosphate buffer range for its complete solution as that required by the amyloid in tissues. It seemed reasonable to consider this fraction as representative of the principal protein component of the amyloid complex. We have not been convinced that the protein fraction B was a dissociation or degradation product of amyloid.

1. Hanssen, O.: *Biochem. Ztschr.* **13**:185, 1908.

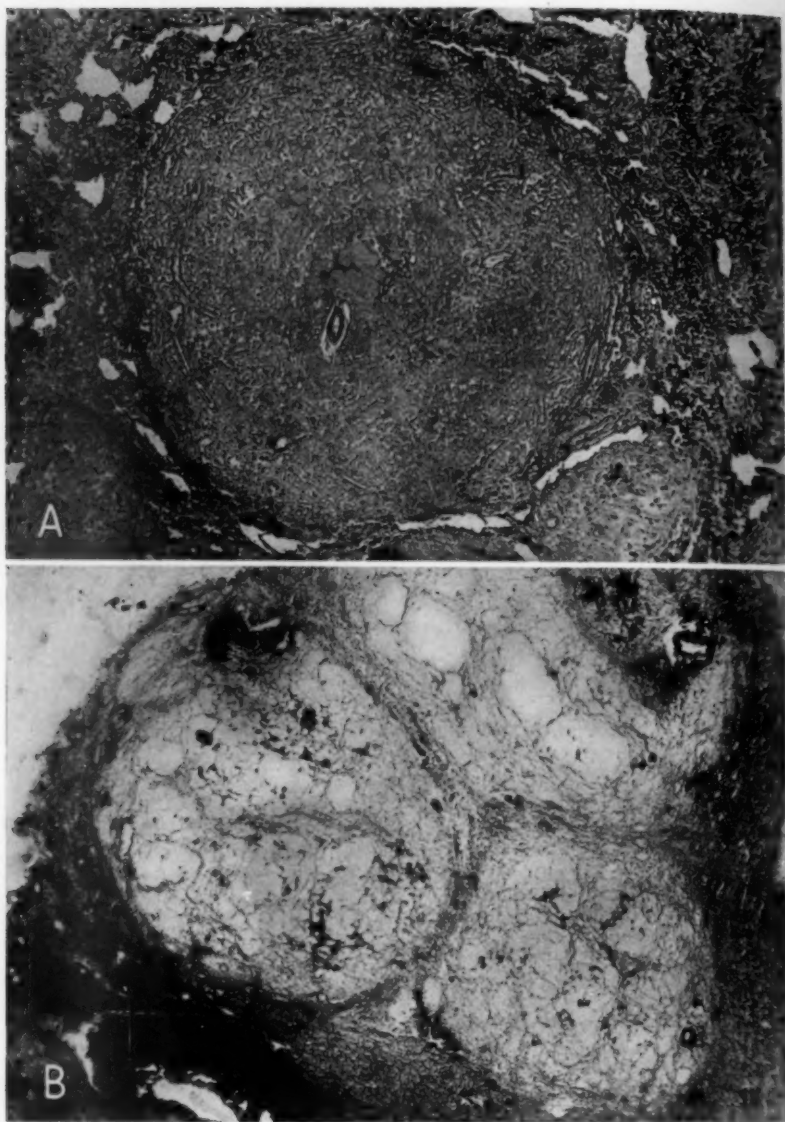


Fig. 3.—*A*, medium power photomicrograph of amyloid deposits in a splenic follicle. Zenker's fixation; phosphotungstic acid-hematoxylin stain. *B*, photomicrograph illustrating the appearance of splenic tissue after complete extraction of amyloid. Parts of three follicles are shown. Note the loss of lymphocytes as well as of amyloid. The disintegration of lymphocytes, although not only always so prominent as indicated in this illustration, interferes with the separation of amyloid protein from splenic tissue protein in the range pH 10 to 11. Zenker's fixation; phosphotungstic acid-hematoxylin stain.

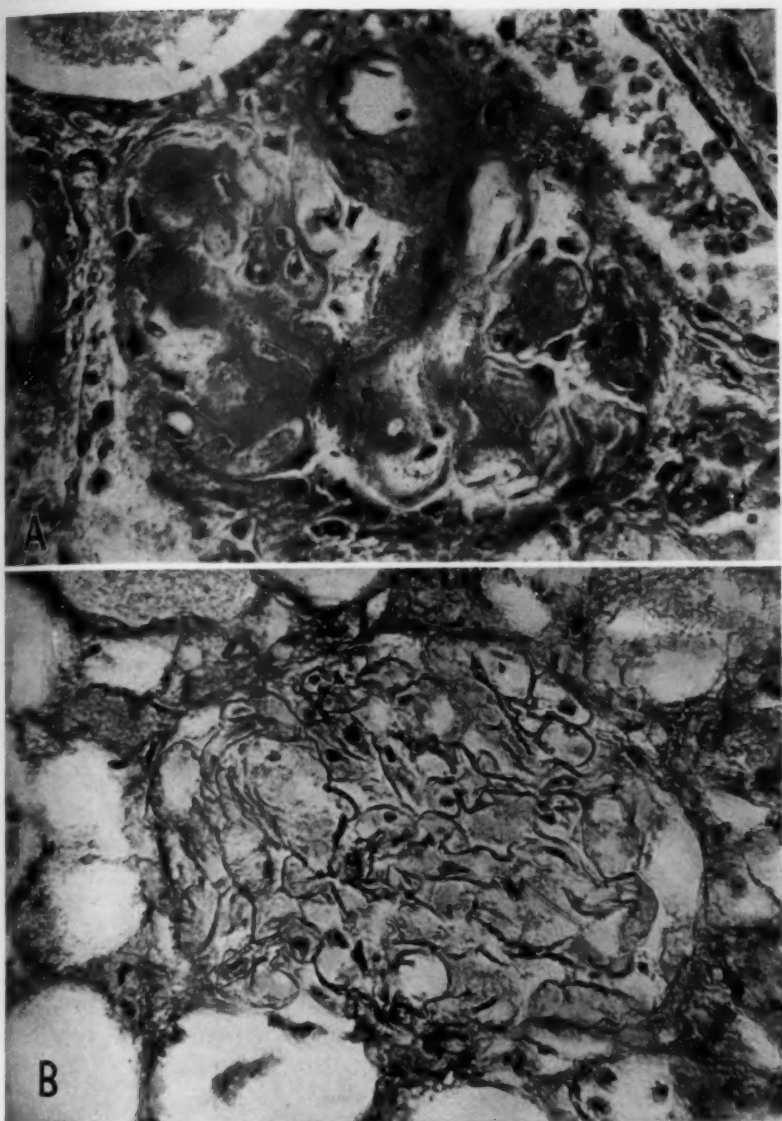


Fig. 4.—*A*, photomicrograph of a renal glomerulus which contains deposits of amyloid. Zenker's fixation; aniline blue stain. *B*, photomicrograph of a glomerulus from the same block as *A* after application of methods for the extraction of amyloid. Zenker's fixation; aniline blue stain.

The study of the proteins by salt precipitation and dialysis yielded the following results: On addition of ammonium sulfate to the neutralized phosphate buffer solvent, a prompt voluminous precipitation of protein occurred. This began at 15 to 17 per cent saturation and was practically complete at 33.3 per cent saturation. The protein fraction so isolated conformed in quantity and general properties to the amyloid fraction A as isolated by acetic acid precipitation. All protein, however, was not precipitated at one-third saturation with ammonium sulfate. The residual protein required one-half saturation with ammonium sulfate for its complete removal from solution. This fraction corresponded roughly to amyloid fraction B, which, as we have noted, was not precipitated by acetic acid at p_H 4 to 5.4.

After centrifugation of the proteins precipitated with ammonium sulfate, some difficulty was occasionally encountered in completely redissolving them in neutral salt solution in preparation for dialysis. This difficulty was attributable in some instances to an overneutralization of the solvent prior to salting out the proteins. Neutralization with carbon dioxide corrected this error. In other instances contaminating lipoids were responsible for the difficulty. Alcohol-ether treatment of the tissues prior to extraction also seemed to alter the proteins so that they were less easily dissolved after salt precipitation. When the amyloid content of the liver was high, and when fatty degeneration of parenchymal cells was minimal, the alcohol-ether treatment was dispensed with. Under these conditions no difficulty was encountered in dissolving the ammonium sulfate precipitate in dilute neutral salt solution.

Dialysis of the proteins precipitated by ammonium sulfate and redispersed in solution at neutrality confirmed the conclusion that there was an essential difference between the two fractions. Fraction A was insoluble in distilled water. Fraction B was soluble. The proportion of the A to the B fraction varied from 10:1 to 50:1. Both fractions were insoluble in 10 per cent sodium chloride at neutrality and at p_H 11.

Fraction A after separation from fraction B by dialysis was soluble in neutral 1 per cent sodium chloride. It was precipitable with acetic acid and after precipitation was soluble in phosphate buffer solutions within the p_H range required for dissolving amyloid from the tissues. The precipitation zone of this product in acetate and phosphate buffers lay between p_H 3.9 and p_H 6.4. The maximum rate and quantity of precipitation occurred at p_H 5.2.

An alkali-soluble protein was present in normal liver with approximately the same properties as amyloid fraction A. This protein, however, was not present in significant amounts in the extracts of normal

liver at p_H 10 to 11, being almost entirely removed below p_H 10. Nevertheless, in the average extraction of amyloid from liver this normal hepatic protein was probably a low percentage contaminant of amyloid fraction A. Similar remarks may be made with respect to amyloid fraction B. Indeed, we have not satisfied ourselves that the low percentage fraction B deserved much consideration as a component of amyloid. On the other hand, we were not justified in disregarding it entirely, because it may represent a significant part of the complex from other than quantitative standpoints.

COMMENT

Amyloid is a pathologic substance. It appears as a homogeneous acidophilic deposit, principally beneath the lining endothelium of capillaries and in the intercellular region of arteriolar walls. It is especially abundant as a rule in localities where reticuloendothelial cells are numerous. It may accumulate in quantities sufficiently great to replace most of the normal cellular structures of an organ. This occurs most frequently in the liver, spleen, adrenal and lymph node.

The deposition of amyloid occurs under comparable conditions in many species. It may accompany numerous pathologic states which at first glance seem to have little in common. The majority of these conditions are chronic infectious diseases, which may or may not be characterized by chronic suppuration.

Amyloid deposition has been produced experimentally in several animal species by repeated injections of bacteria, of sterile bacterial toxins and of nontoxic proteins.² Among the bacteria, staphylococci seem to be organisms of choice for the experimental production of amyloidosis. Among the toxins, diphtheria toxin is very effective, as has been repeatedly shown by the frequent occurrence of amyloid disease in horses used for the production of antitoxin.³ Relatively nontoxic materials such as sodium caseinate, horse serum and human serum will produce amyloid disease in mice if the materials are injected repeatedly over a period of several weeks.⁴ Amyloid frequently appears in the organs of mice which have spontaneous or experimentally induced tumors. Some investigators have claimed that amyloid disease will develop in mice maintained on a diet of nutrose or sodium caseinate.⁵

2. Wells, H. G.: *Chemical Pathology*, ed. 5, Philadelphia, W. B. Saunders Company, 1925.

3. Lewis, P. A.: *J. M. Research* **15**:449, 1906.

4. Jaffé, R. H.: *Arch. Path.* **1**:25, 1926.

5. Kuczynski, M. H.: *Klin. Wchnschr.* **2**:2193, 1923. Smetana, H.: *Bull. Johns Hopkins Hosp.* **37**:383, 1925.

Krawkow,⁶ after treating macerated amyloid-bearing human organs with pepsin and barium hydroxide, isolated a protein which he called an albumose. His studies were not controlled by a similar treatment of normal organs. In addition he obtained a material which had some of the properties of chondroitin-sulfuric acid. He gave no quantitative figures and did not chemically define the composition of the product. Other investigators have not confirmed his results.⁷ The variability among the several analyses may be interpreted in one of two ways. Either amyloid is a product of inconstant composition, or the crude materials prepared for analysis were contaminated with variable amounts of normal tissue elements.

The staining reactions of amyloid need not be discussed at this time. Suffice it to say that the iodine and iodine-sulfuric acid reactions may or may not be present. The aniline dye reactions, such as those with crystal violet and congo red, are more dependable. Indeed, congo red when given intravenously seems to form a fairly stable union with amyloid in the living subject. In this regard we have cited one of our patients who was given an intravenous congo red test two months before death. At autopsy the amyloid in the spleen, kidneys and adrenals was dull red. The dye was extractable from the tissues only within the p_H range at which the amyloid likewise disappeared from the tissue sections.

Most investigators who have studied the dye reactions have expressed the opinion that the observed specificities depend on some labile substance which is set free from the amyloid complex by the action of alkaline solvents or digestive enzymes. Although this may be true, it is discouraging to attempt to analyze the data which have been presented to support the contention. For instance, the "labile" substance of Hanssen which was held responsible for iodine staining was "set free" from the protein of "sago" granules in 0.05 N sodium hydroxide. As we have shown, this happens to be well above the concentration of alkali suitable for dissolving the whole amyloid complex. With the elementary solubility properties of amyloid in mind, some of these problems can now be approached in a rational way.

It is well known that amyloid is often slowly resorbed from the organs of the intact animal. Resorption may follow removal of the condition which led to the deposition of amyloid. The means by which resorption takes place are obscure but are assumed to be enzymatic in nature. There is probably no relationship between the mechanism of physiologic resorption and our radical experimental extraction.

6. Krawkow, N. P.: Arch. f. exper. Path. u. Pharmacol. **40**:195, 1898.

7. Mayeda: Ztschr. f. physiol. Chem. **58**:375, 1909. Eppinger, H.: Biochem. Ztschr. **127**:107, 1922. Hanssen.¹

A survey of the literature, therefore, justifies at least three comments: First, amyloid seems to be a complex of variable composition. Second, amyloid is deposited in elective sites in many conditions which are characterized by prolonged, continuous or repeated stimulation of immune mechanisms. Third, a removal of those stimuli which may be thought of as antigenic may be followed by a spontaneous disappearance of amyloid from the tissues. In the light of present knowledge of amyloidosis, a few instances of amyloid disease do not lie within the range of these comments. As examples, amyloidosis accompanying chronic glomerular nephritis or multiple myeloma may be given.

Many of the facts about amyloid disease lead to the speculation that amyloid may be a product of reaction *in vivo* between antigen and antibody.⁸ If this concept is true, the composition of amyloid should vary in accordance with the nature of the antigen and of the homologous antibody. Furthermore, it might be predicted that there would be similarities between amyloid, regarded as an antigen-antibody complex, and the corresponding complex formed *in vitro*. There are two reasons for believing that the two complexes would not be identical. First, amyloid is deposited slowly over a long period of time in a changing environment, while specific precipitates *in vitro* are deposited rapidly under standard simplified conditions. Second, circulating chemical compounds may enter fortuitously into combinations with amyloid, once it has been deposited. A good example of such an addition to the amyloid matrix is afforded by the entrance of circulating congo red into amyloid. The resultant dye-amyloid compound persists long after congo red has disappeared from the circulation and, as we have shown, has properties different from those possessed by amyloid prior to its conjunction with the dye. Congo red, so far as its ordinary chemical properties are concerned, is not unique, and so it seems reasonable to believe that naturally occurring compounds with similar reactive groups may unite with the amyloid matrix in a comparable way. Hence, the isolation of a compound, antigenic or otherwise, from the amyloid matrix does not necessarily indicate that the compound played an essential role in the formation of the matrix in question.

In view of this difficulty in the interpretation of the significance of small quantities of material in amyloid, and because of the high proportion of antibody protein in antigen-antibody precipitates, it seems desirable to test the given hypothesis by attempts to isolate antibody protein from the amyloid matrix. Unfortunately, there is no satisfactory method for dissociating specific precipitates composed of protein antigen

8. Letterer, E.: *Virchows Arch. f. path. Anat.* **293**:34, 1934.

and protein antibody. For this reason it is improbable that the two proteins obtained by our methods represent unimpaired dissociated antigen and antibody. Nevertheless, we tried to find conditions under which the two proteins would unite to form an insoluble precipitate possessing the properties of amyloid matrix. Thus far our efforts have been unsuccessful.

Although elementary properties of protein-specific precipitates have not been systematically studied, Heidelberg and Kendall⁹ found that an azoprotein antigen-antibody precipitate in which they were interested was soluble in 0.02 N sodium hydroxide. It happens that this concentration of sodium hydroxide is likewise suitable for dissolving amyloid from tissues.

Felton¹⁰ demonstrated dissociation of pneumococcus antibody-polysaccharide precipitates at p_H 9 to 9.6 in solutions composed of hydroxides and phosphates of strontium or calcium. Heidelberg and Kabat¹¹ proved that similar complexes dissociate in neutral aqueous 15 per cent sodium chloride solution, although they employed a modification of Felton's alkali dissociation method for dealing with residual insoluble precipitate. In connection with these results it is to be recalled that amyloid is refractory to the action of alkaline phosphate buffer solutions with a p_H of less than 10 and that concentrated salt solutions have no demonstrable effect on the composition of amyloid.

Our experiments were not specifically designed for approaching the particular problem which has been discussed in the preceding paragraphs. We were primarily interested in devising a simple method for studying a few elementary properties of amyloid irrespective of its locality in tissues or the disease with which it is associated. In the particular type of amyloidosis which served as the subject for our investigation the sites of amyloid deposition and the disease which was assumed to be responsible for the amyloidosis were constant factors. Nevertheless, three varieties of amyloid were detected. The usual type corresponded with that which is commonly described in generalized amyloidosis. The properties of this amyloid were uniform and were independent of visceral location or variations in the type of pulmonary tuberculosis. The second variety of amyloid differed from the usual type in that it was present only in the liver and possessed the property of nonhomogeneous birefringence. This property disappeared during extraction of the sections with buffer solutions which were less alkaline than those required for dissolving the matrix. It did not reappear on subsequent acidification of the tissue

9. Heidelberg, M., and Kendall, F. E.: *J. Exper. Med.* **62**:697, 1935.

10. Felton, L. D.: *J. Immunol.* **22**:453, 1932.

11. Heidelberg, M., and Kabat, E. A.: *J. Exper. Med.* **67**:181, 1938.

sections. From these data it may be concluded that the substance responsible for the property was in all likelihood set free from the matrix at p_H 9.5 to 10. The third type of amyloid was a congo red-amyloid complex. This was less soluble than the usual type of amyloid in alkaline buffer solutions. This dye-amyloid complex in a sense is artificial. It is emphasized, however, because it is a visible illustration of the capacity of amyloid matrix for combining with a circulating chemical compound. That the compound in this instance happened to be congo red is beside the point, for, theoretically, compounds with similar general properties should combine with the matrix in the same way. In this regard it is pertinent to mention the observation that congo red was completely separated from the protein matrix, if at all, only in the p_H range required for solution of the matrix.

All three types of amyloid, despite their differences in composition, disappeared from the tissues in phosphate buffer solutions at p_H 11 and remained in the tissues in phosphate buffer solutions at p_H 10. As amyloid disappeared from the tissues, two proteins appeared in the solvent. One protein comprised about 90 per cent of the total protein. The second protein, which was always present in small amounts, had different properties. Normal liver and spleen possessed similar alkali-soluble proteins. Therefore, for purposes of purification it was necessary to determine a range of p_H within which there would be a minimal contamination of amyloid protein with normal tissue protein. Fortunately, almost all normal liver protein soluble in the range p_H 7 to 11 is soluble in the range p_H 7 to 10. By a preliminary extraction of amyloid-bearing liver at p_H 10 it was possible to remove most normal proteins, and by subsequent extraction at p_H 11, to obtain amyloid proteins in reasonably pure form. This method was not effective when amyloid-bearing spleen was used, because a large amount of normal splenic protein is soluble in the range p_H 10 to 11.

The minimum p_H , about p_H 11, which was required for dissolving amyloid is an interesting value from several points of view. It is probable that this degree of alkalinity was insufficient for bringing about any radical changes in either one of the proteins which were isolated. Most pure proteins are fairly stable, at least for short periods, in buffer solutions at p_H 11. Many conjugated proteins, however, such as most glycoproteins and nucleoproteins, are altered under conditions similar to those employed in our experiments. These alterations consist primarily in a separation of the proteins for the prosthetic groups.¹² Thus, by

12. Levene, P. A.: *Hexosamines and Mucoproteins*, New York, Longmans, Green & Company, 1925. Levene, P. A., and Bass, L. W.: *Nucleic Acids*, American Chemical Society Monograph Series, no. 56, New York, The Chemical Catalog Company, Inc., 1931.

appropriate manipulations subsequent to exposure of such compounds to alkaline mediums the protein and the nonprotein constituents can be recovered from the solvent. If the prosthetic group is responsible for the insolubility of the complex in neutral mediums, the separation of the two components frequently results in the recovery of a soluble protein and a soluble prosthetic group. From a quantitative point of view the nonprotein component of such a complex may be relatively insignificant. It is for these reasons that future investigations of amyloid might well be concerned with the possibility that some nonprotein component is set free from the matrix at p_H 11, thus permitting the protein to pass into solution. It seems, however, that this concept introduces unnecessary complications, because the principal protein of amyloid after precipitation with acetic acid at p_H 4 to 5.4 is not wholly soluble in solutions less alkaline than those required for dissolving amyloid from tissues. This may mean that the fundamental solubility property of amyloid is a function solely of the protein and is independent of degradation of a hypothetical complex. In this regard it is of interest that many pure proteins bind sodium hydroxide in large amounts in the neighborhood of p_H 11. Proteins insoluble at neutrality may thereby become soluble at p_H 11 as sodium salts and retain their solubility as such on neutralization of the medium. It seems that the principal protein fraction of amyloid behaves in this manner, but this is likewise true of the protein component of some nucleoproteins, and hence is not a valid argument in favor of a concept which recognizes the principal fraction as the only component of amyloid.

With these theoretic considerations and experimental data in mind, it might be useful to construct a model of the amyloid matrix. The fundamental unit in this structure is the protein fraction A. It may be assumed that this fraction in the tissues is in a state similar to that brought about by acetic acid precipitation in vitro. Bound up in some way with this fraction is a minor questionable component, protein fraction B. Inasmuch as fraction B is incapable of combining with fraction A to form an insoluble precipitate at neutrality and inasmuch as we believe that amyloid is a precipitate which forms at physiologic neutrality in the tissues, an unknown component which acts on fraction A in vivo in a way comparable with that of acetic acid in vitro is a desirable part of the model. In addition to these primary constituents the matrix must possess residual affinities by which certain circulating substances are temporarily or permanently bound in the matrix. By virtue of this property the matrix becomes subject to various additions and substitutions. These alterations may be thought of as less dependent on conditions essential for the formation of the matrix than on the composition of the fluids with which the matrix is in contact after its appearance as a formed substance in the tissues.

SUMMARY

Amyloid-bearing tissues obtained post mortem from patients with chronic pulmonary tuberculosis were cut into thin sections with a freezing microtome. Three types of amyloid were encountered. All were insoluble in buffer solutions ranging from p_H 1 to 10. The three varieties, whether present in the liver, spleen or kidney, were soluble at 5 to 10 C. in phosphate buffer solution at p_H 11. Almost all normal liver protein soluble in the range p_H 7 to 11 was soluble in the range p_H 7 to 10. By successive extractions of thin sections of fresh amyloid-bearing liver at p_H 7, p_H 10 and finally p_H 11 it was possible to obtain hepatic amyloid protein in a reasonably pure form. Two fractions of protein were obtained. The principal fraction amounted to 85 to 90 per cent of the total protein extracted at p_H 11. It remained in solution on neutralization of the solvent. At 15 to 17 per cent saturation with ammonium sulfate, on addition of acetic acid or on dialysis against distilled water it began to precipitate, and at half saturation with ammonium sulfate was completely precipitated. The second protein fraction remained in solution on neutralization of the alkaline buffer solvent. It was precipitated along with the principal fraction by half saturation of the solvent with ammonium sulfate but was soluble in dilute acetic acid and distilled water.

GENESIS OF HYDATIDIFORM MOLE

ARTHUR T. HERTIG, M.D.

AND

HENRY W. EDMONDS, M.D.

BOSTON

It is the purpose of this paper to emphasize the origin, and to trace the development, of the typical hydatidiform mole of pregnancy. During the past seven years the interest of one of us (A. T. H.) has been stimulated by this problem by virtue of contact with a large volume of pathologic material obtained in cases of spontaneous abortion. Material of this sort was first seen in significant amounts during a year spent at the Carnegie Institution of Washington, in the Department of Embryology, under the direction of Dr. George L. Streeter. There, for the first time, the senior author became aware of the frequency of hydatidiform degeneration in early abortuses, especially the pathologic ones in which the embryo was either absent or very defective. Dr. Franklin P. Mall, the founder of that laboratory, and his co-author Dr. A. W. Meyer pointed out in 1921¹ the frequency of this condition and stressed its relationship to the typical mature hydatidiform mole of later pregnancy. In spite of this classic work and others to be reviewed later, the medical profession in general still regards hydatidiform degeneration of the chorion (placenta) as a rare disease, as attested by the relatively large number of case reports which still appear in current obstetric literature.

It is quite true that the typical hydatidiform mole of pregnancy is a relatively uncommon disease, as shown by the incidence in our own clinic, namely, 1 in 2,062 deliveries (Irving²) over a period of twenty years. This is probably a fairly accurate index of the incidence of this phase of the condition and tends to be confirmed by the figures of another large American clinic, namely, the New York Lying-in Hospital, where

From the Laboratory of Pathology the Boston Lying-in Hospital and the Departments of Obstetrics and Pathology, Harvard Medical School.

This investigation has been aided by a grant from the Carnegie Corporation of New York to study hydatidiform degeneration of the placenta and allied problems in embryology. This study, started in 1935, has been aided also by a grant from the Wellington Fund, of the Harvard Medical School.

1. Mall, F. P., and Meyer, A. W.: *Contrib. Embryol.* (no. 56) **12**:203, 1921.
2. Irving, F. C.: *Textbook of Obstetrics*, New York, The Macmillan Company, 1936.

the incidence is 1 in 2,334 deliveries (Sherman³) occurring over a period of thirty-four years. In the world literature, however, Das⁴ found that the incidence varied from 1:240 to 1:20,000 pregnancies. This discrepancy is probably due mainly to differences in various authors' criteria as to what constitutes a hydatidiform mole, although other factors undoubtedly help to account for this variation.

The classic manifestation of this disease, with the chorion replaced by masses of grapelike vesicles varying from 1 to 25 mm. in diameter, needs no further description. The aspect of the disease that does need emphasis and elaboration is that of its origin and evolution from the typical, common early pathologic or "blighted" ovum, which constitutes the abortus in almost 50 per cent of cases of spontaneous abortion and shows frequent early hydatidiform degeneration of its chorionic villi, to the uncommon classic form of the disease that has been known for centuries.

Drs. Frederick C. Irving and George L. Streeter, by encouragement and advice, were responsible for our undertaking this study of the pathologic nature of spontaneous abortion, of which the present communication is but one phase. Dr. S. Burt Wolbach gave to one of us (A. T. H.) help and advice on this and allied problems. Many associates have contributed material, without which this study would have been impossible.

REVIEW OF THE LITERATURE

For nearly a hundred years it has been noted by various authors that mild hydatidiform degeneration of the placenta is a common occurrence in early abortuses but that a marked degree of this degeneration, resulting in the classic picture of a hydatidiform mole in the middle trimester of pregnancy, is rare. Thus, Boivin⁵ in 1827 stated that the latter condition occurred once in 20,000 pregnancies. A few of the early authors, notably Gierse⁶ and Storch,⁷ emphasized that there exist transitional stages between these two forms. However, Mall and Meyer,¹ who, in a classic monograph on abortuses, exhaustively reviewed the literature on this subject since 1847, stated: "No one seems to have followed its [hydatidiform degeneration] evolution, although hydatidiform degeneration, whether total or partial, is of course gradual in its advent." That such a concept of the "evolution" of this common and occasionally dangerous complication of pregnancy is still

3. Sherman, J. T.: *Am. J. Surg.* **27**:237, 1935.

4. Das, P. C.: *J. Obst. & Gynaec. Brit. Emp.* **45**:265, 1938.

5. Boivin, M. A.: *Nouvelles recherches sur la nature, l'origine et le traitement de la môle vésiculaire ou grossesse hydatique*, Paris, Méquignon l'aîné père, 1827.

6. Gierse, A.: *Verhandl. d. Gesellsch. f. Geburtsh. in Berlin* **2**:126, 1847; cited by Mall and Meyer.¹

7. Storch, E. D.: *Virchows Arch. f. path. Anat.* **72**:582, 1878.

lacking from the literature on the subject is attested by recent reviews (Mathieu⁸) and statistical analyses (Das⁴). Schumann,⁹ in his recent "Textbook of Obstetrics," also emphasized the lack of a clearly defined theory of the genesis of hydatidiform mole by quoting the statement from the monograph of Mall and Meyer given here.

From time to time various papers on the causes of hydatidiform degeneration of the chorion have appeared, such as those of Hinselmann,¹⁰ Kleine,¹¹ Haas¹² and others. Mall and Meyer,¹ after carefully sifting the evidence in the literature as well as that gleaned from their own extensive material in the Carnegie Embryological Collection, concluded that endometritis played a prominent etiologic role. This possible factor was mentioned as early as 1863 by Virchow¹³ and from then on to modern times by such authorities as Lwow,¹⁴ Emanuel,¹⁵ Marchand,¹⁶ Veit,¹⁷ Essen-Möller¹⁸ and others, including DeLee.¹⁹ There were some, however, including Taussig,²⁰ who stressed that mere leukocytic infiltration of the decidua did not constitute infection. Perhaps the best single argument that endometritis is not the primary etiologic agent responsible for hydatidiform degeneration is the occurrence of segmental areas of this condition in an otherwise normal placenta (Thaisz²¹) associated with a normal full term infant. In line with this general thesis are the instances of double ovum, twin pregnancies in which one ovum shows hydatidiform degeneration of the chorion (usually without a

8. Mathieu, A.: *Internat. Abstr. Surg.* **68**:52, 1939; in *Surg., Gynec. & Obst.*, January 1939.

9. Schumann, E. A.: *Textbook of Obstetrics*, Philadelphia, W. B. Saunders Company, 1936.

10. Hinselmann, H.: *Zentralbl. f. Gynäk.* **55**:261, 1931.

11. Kleine, H. O.: *Arch. f. Gynäk.* **145**:261, 1931.

12. Haas, A.: *Med. Klin.* **21**:811, 1925.

13. Virchow, R.: *Die krankhaften Geschwülste*, Berlin, A. Hirschwald, 1863, vol. 1, pp. 405-414.

14. Lwow, J. M.: Abstracted, *Centralbl. f. Gynäk.* **16**:20, 1892; cited by Mall and Meyer.¹

15. Emanuel, R.: *Ztschr. f. Geburtsh. u. Gynäk.* **31**:1875, 1894; cited by Mall and Meyer.¹

16. Marchand, F.: *Ztschr. f. Geburtsh. u. Gynäk.* **32**:405, 1895; cited by Mall and Meyer.¹

17. Veit, J.: *Handbuch der Gynäkologie*, Wiesbaden, J. F. Bergmann, 1899, vol. 3; cited by Mall and Meyer.¹

18. Essen-Möller, E.: *Studien über Blasenmole*, Wiesbaden, J. F. Bergmann, 1912; cited by Mall & Meyer.¹

19. DeLee, J. B.: *Principles and Practice of Obstetrics*, ed. 2, Philadelphia, W. B. Saunders Company, 1915.

20. Taussig, F. J.: *Weekly Bull. St. Louis M. Soc.* **5**:79, 1911; cited by Mall and Meyer.¹

21. Thaisz, K.: *Zentralbl. f. Gynäk.* **62**:1937, 1938.

fetus) while the other ovum consists of a normally formed fetus (often living and normally delivered) enclosed within a normal chorion. The literature on this aspect of hydatidiform degeneration has recently (1938) been reviewed by Strauch.²² Aside from this critical evidence against such an etiologic factor, we feel, on the basis of evidence not yet published, that endometritis plays no etiologic role in the usual hydatidiform degeneration, since in the vast majority of cases of spontaneous abortion and miscarriage, regardless of whether the chorion shows hydatidiform degeneration or not, there is more or less evidence of inflammation and occasionally of frank infection of the decidua.

It has long been noted (Hewitt,²³ Hahn²⁴ and others) that there is a close correlation (at least in time) between hydatidiform degeneration and the disappearance of blood vessels in the chorionic villi thus affected. Whether this disappearance causes hydatidiform change (and follows the death of the embryo), as held by Hewitt,²³ or is merely the first recognizable change in this degenerative process, as suggested by Mall and Meyer,¹ has not been settled. The latter authors suggested that there was evidence that in some instances hydatidiform degeneration may even begin before the villi have become vascularized. Recent work by one of us (A. T. H.)²⁵ has shown, however, that chorionic villi in man and in *Macaca mulatta* develop simultaneously with their blood vessels. Hence, it appears that regardless of when this degenerative process starts it must, of necessity, take place in villi that at least contain vascular primordia. All writers on the subject, however, have been in agreement as to the avascularity of the typical cystic or hydatidiform villi. Hinselmann,¹⁰ as well as Keller and Adrian,²⁶ favor a disturbance or an alteration in the vascularity of the chorionic villus as the cause of hydatidiform degeneration thereof. Because of these changes in the growth of the chorionic vessels there accumulates within the villus fluid which in the normal course of events (i. e., proper functioning of the chorionic circulation) would have been carried away. With this accumulation of fluid, the laws of hydrostatics influence the formation of a typical cystic villus. There is secondary epithelial proliferation in response to this distention. This is in disagreement with the earlier writers, notably Marchand,¹⁰ who maintained that the epithelial proliferation was primary.

The age and parity of the patient have been mentioned by various authors as etiologic factors. The latest views on the subject, however,

22. Strauch, H.: *Zentralbl. f. Gynäk.* **62**:1371, 1938.

23. Hewitt, G.: *Tr. Obst. Soc., London* **1**:248, 1860.

24. Hahn, C. F. O.: *Monatschr. f. Geburtsh. u. Frauenkrankh.* **26**:33, 1865.

25. Hertig, A. T.: *Contrib. Embryol. (no. 146)* **25**:37, 1935.

26. Keller, R., and Adrian, J.: *Gynec. et obst.* **38**:332, 1938.

based on a statistical analysis of 846 cases in the world literature by Das,⁴ seem to indicate that the hydatidiform mole may occur at any age and at any stage in the patient's child-bearing period. He found that in Indian women (a series of 40 cases) 45 per cent of the moles occurred between the ages of 21 and 30, in contrast to a stated occurrence of 40 per cent for the same decade in the world literature. The decades immediately preceding and following this showed an equal occurrence for Indian women of 22.5 per cent, whereas after the third decade there was a gradual decline in the occurrence of these abnormal pregnancies in European women. This difference was accounted for on the basis of early marriage among Indian women. This author also found that, while most of the women who delivered moles were multiparas, 21.4 per cent were primiparas and 10 to 18 per cent were primigravidas. In our own series the mean gravidity is 2.5 and the mean parity is 1.1 (data available in 53 cases). Mall and Meyer¹ pointed out that hydatidiform moles are relatively more common after the age of 40, i. e., approximately one fourth of the moles reported up to that date (1921) occurred in the decades during which only one tenth of the children were born. Nevertheless, the average age of patients producing hydatidiform moles, according to these authors, is 29.6 years, which is in close accordance with the 28.9 years reported in the statistical analysis by Das⁴ and with a mean age of 28.9 years for 73 such patients in our own series.

The association of the theca lutein cysts with some hydatidiform moles (11 per cent, according to Mathieu⁶ in 1939) gave rise to speculation among earlier authors, such as Haas,¹² Pick,²⁷ Stoeckel²⁸ and others, as to whether these ovarian changes were primary or secondary. Sherman,³ in 1935, summed up modern thought on this aspect of the etiologic controversy by stating: "If lutein cystomata is the cause [of hydatidiform degeneration of the placenta] and not the result, the relationship of the hormones in the sexual cycle, at present believed to be the most logical conception, cannot be accepted."

MATERIAL AND METHODS

Following the year spent by one of us (A. T. H.) with Dr. Streeter in studying the embryologic aspects of spontaneous abortion, a program was undertaken at the Boston Lying-in Hospital to build up a collection of spontaneously aborted ova. Such material, sought from all over New England, is examined grossly and microscopically from both an embryologic and a pathologic angle. A binocular dissecting microscope, with powers of magnification up to 100 diameters, is used to facilitate

27. Pick, L.: *Berl. klin. Wchnschr.* **34**:1069 and 1097, 1897.

28. Stoeckel, W., in *Beiträge zur Geburtshilfe und Gynäkologie*. Festschrift Professor Dr. Heinrich Fritsch bei Gelegenheit des 25 jährigen Bestehens des Centralblatts für Gynäkologie in Dankbarkeit und Verehrung gewidmet von seinen Schülern, 136, Leipzig, Breitkopf u. Härtel, 1902.

the examination of the specimen in the gross. A full report of the observations is returned to the donor of the material together with, if possible, an interpretation of any etiologic factors involved in the abortion. In this way it was hoped that over a period of time it would be possible to assemble data that might shed some light on the causes and the genesis of the lesions responsible for spontaneous abortion.

From September 1934 through December 1939 there have been received for pathologic examination the products of conception in 1,027 cases of spontaneous abortion and miscarriage. During this period of somewhat more than five years the number of specimens thus examined has averaged approximately 200 a year. Many of these have been submitted by physicians in Boston, although moderate numbers of specimens have been received from hospitals and physicians in other communities. Since a good many of the physicians on the staff of the Boston Lying-in Hospital submit every spontaneously aborted ovum from their private practices, it is felt that this material is truly representative of the variety of pathologic conditions associated with spontaneous abortion in general.

Early in this study one of us (A. T. H.) became interested in the possibility of a correlation between the microscopic appearances of hydatidiform mole and the clinical outcome for the patient. Because of the scarcity of typical molar pregnancies in any one clinic (there were only 19 in the Boston Lying-in Hospital during the period from 1916 to 1935) it was decided to obtain tissue from a large enough series of cases so that it could be determined statistically whether there was or was not any such correlation. This phase of the study was further stimulated by the statements of various authors that the pathologic examination of any given mole gave no indication as to whether it was benign or malignant.

A preliminary report, in which the first 24 cases of molar pregnancy were analyzed and the morphologic aspects of the ova correlated with the clinical outcome, was presented by one of us (A. T. H.) before the Massachusetts Medical Society in discussing a paper given by Dr. L. E. Phaneuf²⁹ entitled "Hydatidiform Mole and Chorionepithelioma."

During the past five years tissues from approximately 125 cases of hydatidiform mole have been accumulated, although only 74 cases have thus far been studied. These cases are not included in those of spontaneous abortion cited in a foregoing paragraph, although some of the information gained from their study is contained in this paper.

CLASSIFICATION OF THE MATERIAL

The classification followed in studying these abortuses is essentially the same as that devised by Mall (Mall and Meyer¹). Pathologic ova in general are those in which the embryos are absent, very defective or macerated. Normal ova are those in which the embryos are normal or possess only localized anomalies. Normal embryos may be in abnormal chorions, and vice versa.

The pathologic ova are classified in more detail as follows:

Group 1: Villi Only.—This material contains only chorionic villi, whether normal or abnormal. Obviously this group is one of convenience only, since it merely classifies the material submitted, which may or may not adequately represent the relationship between the

29. Phaneuf, L. E.: New England J. Med. **217**:770, 1937.

maternal and the ovular tissues. This includes, therefore, curettings in cases of incomplete abortion. Cases of ectopic pregnancy show a preponderance of this type of ovum; such cases are not included in this study. Logically, this group includes most true hydatidiform moles, since the grapelike cysts are derivatives of the chorionic villi. Usually the typical specimen is without evident traces of the chorionic membrane when it is submitted to the pathologist. For the purpose of this paper we are not including the moles in this category, since our main thesis is to trace the development of moles from the chorionic villi of pathologic ova. To classify moles thus would be somewhat analogous to using in a definition of a word the word one is attempting to define.

Group 2: Empty Chorionic Vesicle.—This type of specimen when intact represents the most pathologic type of ovum with which the pathologist has to deal. There is no derivative of the inner cell mass, i. e., of the portion of the fertilized egg destined to form the embryo. If the chorion is ruptured, one might have reasonable doubt about the essential pathologic nature of the ovum; i. e., the normal embryo with its surrounding amnion may have been lost during the abortion. However, if trauma has produced such an artefact in an otherwise normal ovum, one can usually see evidence of the torn stump of a normal umbilical cord with its radiating vessels.

Group 3: Chorion Containing Empty Amnion.—This type of ovum is only slightly less pathologic than the previous one, there being no evidence of an embryo, although the amnion is present. Members of this group are likewise valid if intact—as they often are—although if ruptured, erstwhile normal ova with the embryos missing can usually be detected and differentiated from true group 3 specimens.

Group 4: Chorion and Amnion Containing Nodular Embryo.—This type is truly pathologic, as the embryonic mass consists merely of a disorganized group of embryonic cells. Artefacts in the group would consist of the macerated remains of an otherwise normal umbilical cord within either a ruptured or an intact amnion.

Group 5: Chorion and Amnion Containing Cylindric Embryo.—If the head end of the embryo can be recognized, even though it does not possess any other features of an embryo, such a specimen is valid for this group. (It is quite rare in our experience.)

Group 6: Chorion and Amnion Containing Stunted Embryo.—It is possible to recognize the embryonic form, although it is much smaller than it should be for the menstrual age of the specimen. One or more portions of the embryo are atrophic, deformed or degenerated. This is a valid group whether the chorion and amnion are ruptured or not, since the embryo has to be recognized before the specimen may be placed in this category.

Group 7: Chorion and Amnion Containing Macerated Embryo.—

These embryos are normal except for maceration or other evidences of change following intrauterine death. Obviously, these specimens started out as normal ova but any one of a number of things may have happened to cause their death, thereby allowing maceration with its sequelae to become evident. In our laboratory this group is used merely for purposes of broad classification, since we attempt to find the pathologic condition responsible for the death of the embryo and hence the cause of the abortion.

Because of the fact that the presence of a ruptured chorionic sac might introduce an element of error in determining whether an ovum is pathologic or not, we have further divided groups 2 to 6 into sub-

TABLE 1.—*Abortuses Examined During 1934-1939*

Year	Pathologic Ova Groups 1-6	"Nonpathologic Ova"		Total
		Group 7 (Macerated Embryo)	Miscellaneous*	
1934-1935.....	81	63	50	194
1936.....	94	61	48	193
1937.....	106	69	44	219
1938.....	105	48	48	201
1939.....	111	42	67	220
Total.....	487	283	257	1,027
Percentage.....	47.4	27.6	25	100

* This group includes a few specimens consisting of decidua of pregnancy or masses of obviously normal placental tissue removed by curettage in cases of incomplete abortion; the large majority of the specimens, however, contained normal embryos. In the cases of incomplete abortion the patients usually gave a history of having passed an embryo which was not submitted for examination.

groups, designated as A and B, indicating a ruptured and an unruptured chorionic sac, respectively.

In table 1 is recorded the number of spontaneously aborted ova examined in each broad group during each year of this study.

The group of "nonpathologic ova" have only one thing in common: the embryo is normal (but often macerated), although it may occasionally possess a localized anomaly not necessarily incompatible with full term development of the ovum. Examples of such localized anomalies are spina bifida, meningocele, hydrocephalus, anencephaly and deformed limb buds. In the first series of 219 cases of spontaneous abortion analyzed, the ratio of pathologic to "nonpathologic" ova was not significantly different from the corresponding ratio in the larger series in table 1. The following list gives the principal causes of the abortion in the 54 per cent of this smaller series in which the abortuses were classified as "nonpathologic" ova.

No Defects

- 4 % Normal embryos—abortions criminally induced (or possibly so)

Ovular Defects

- 6 % Normal embryos with localized anomalies
(spina bifida, meningoencephalocele, etc.)
1.5 % Placenta membranacea
10 % Placenta circumvallata—complete
2 % Placenta diffusely infarcted
4 % Placenta disproportionately small
0.5 % Cord about the neck

Maternal Defects

- 9 % Low implantation
3.5 % Bacterial inflammation of decidua—acute
1.5 % Fetal septicemia
0.5 % Pyelitis
1 % Appendicitis with peritonitis
1 % Chronic endometritis (placenta accreta in one of these cases)

Miscellaneous

- 0.5 % Heavy irradiation (resulting in microcephalic monster)
0.5 % Faulty implantation

Unclassified—Normal Ova

- 8.5 % Hemorrhage into the placenta or into and on the decidua associated with the abortion in most of the cases

STAGES IN THE GENESIS OF HYDATIDIFORM DEGENERATION

Early Stage as Seen in Normal and Pathologic Ova.—It is the essentially pathologic ovum, of which 487 specimens have been examined during the period covered in this study, that is most closely related to the genesis of hydatidiform degeneration, since the initial stages in the process are most frequently found in this type of abortus. The remainder of this section of the paper will be devoted to describing these stages of hydatidiform degeneration. Mention will be made of early stages of hydatidiform degeneration occurring in normal ova.

(a) *Age of Pathologic Ova at Time of Abortion:* In a consecutive series of 53 cases of abortion in which pathologic ova of groups 2 to 6, inclusive, were obtained, in 1936, from women the exact dates of whose last menstrual periods were known, the mean duration of pregnancy was ten and two tenths weeks. The duration ranged from five and five tenths to fifteen and five tenths weeks. This is in close agreement with the universal clinical impression that spontaneous abortion occurs during the second and third months of pregnancy in most cases; a pathologic ovum constitutes the specimen in 47.4 per cent of these cases.

(b) Normal Ovum of Age Comparable to That of Aborted Pathologic Ova: In order to demonstrate how these pathologic ova differ in appearance from normal ova of their general age group, figure 1 has been prepared to show the essential gross and microscopic features of a normal eight week ovum removed by therapeutic hysterectomy. The chorion laeve with overlying decidua capsularis has been removed to reveal the normal 18 mm. embryo within its amnion, (fig. 1 *A*). The cut edge of the chorion frondosum is seen toward "2 o'clock." One of the typical villi in that region was excised and photographed under fluid (fig. 1 *B*). It is clearly evident that this structure has a main stem with four main branches—each giving rise by dichotomous branching to subsidiary branches. The essentially uniform caliber of each of these various portions within its own limits is readily seen. Figure 1 *C* gives the essential microscopic features of villi from such an ovum. The chorionic epithelium is luxuriant and typically double layered. The stroma is loose and of immature fibroblastic type, and contains thin-walled capillaries, enclosing nucleated and non-nucleated red cells.

(c) Early Hydatidiform Degeneration of the Chorion Laeve of the Normal Ovum: Early stages of what appears to be hydatidiform degeneration not infrequently occur in the villi on the chorion laeve of the normal ovum. This is most evident at eight to twelve weeks, when the chorion laeve is normally undergoing atrophy and degeneration incident to the growth of the ovum, with resultant bulging of the latter into the uterine cavity. That this phenomenon is significant with respect to the whole matter of hydatidiform degeneration is quite probable. It engaged the attention of Muggia³⁰ in 1915, who concluded that its alleged occurrence in a small series of reported cases represented merely a local area of hydatidiform degeneration of the placenta. This subject is under investigation at present in our laboratory. Beyond the facts that it does occur, that it is localized to the chorion laeve in the normal ovum and that it is identical grossly and microscopically with early hydatidiform degeneration of pathologic ova, we are not prepared to go. Figure 2 represents a low power photomicrograph of such an area, discovered with the binocular dissecting microscope under a magnification of 10 diameters. The loose fibroblastic stroma shows great accentuation of the intercellular spaces by the accumulation of finely granular basophilic precipitate. The vessels have disappeared or are degenerating, while the chorionic epithelium remains essentially normal in appearance.

(d) Early Hydatid Degeneration in Pathologic Ova: Of 487 spontaneously aborted ova examined during a period of five years and four

30. Muggia, V.: *Folia gynaec.* 11:347, 1915; cited by Mall and Meyer.¹

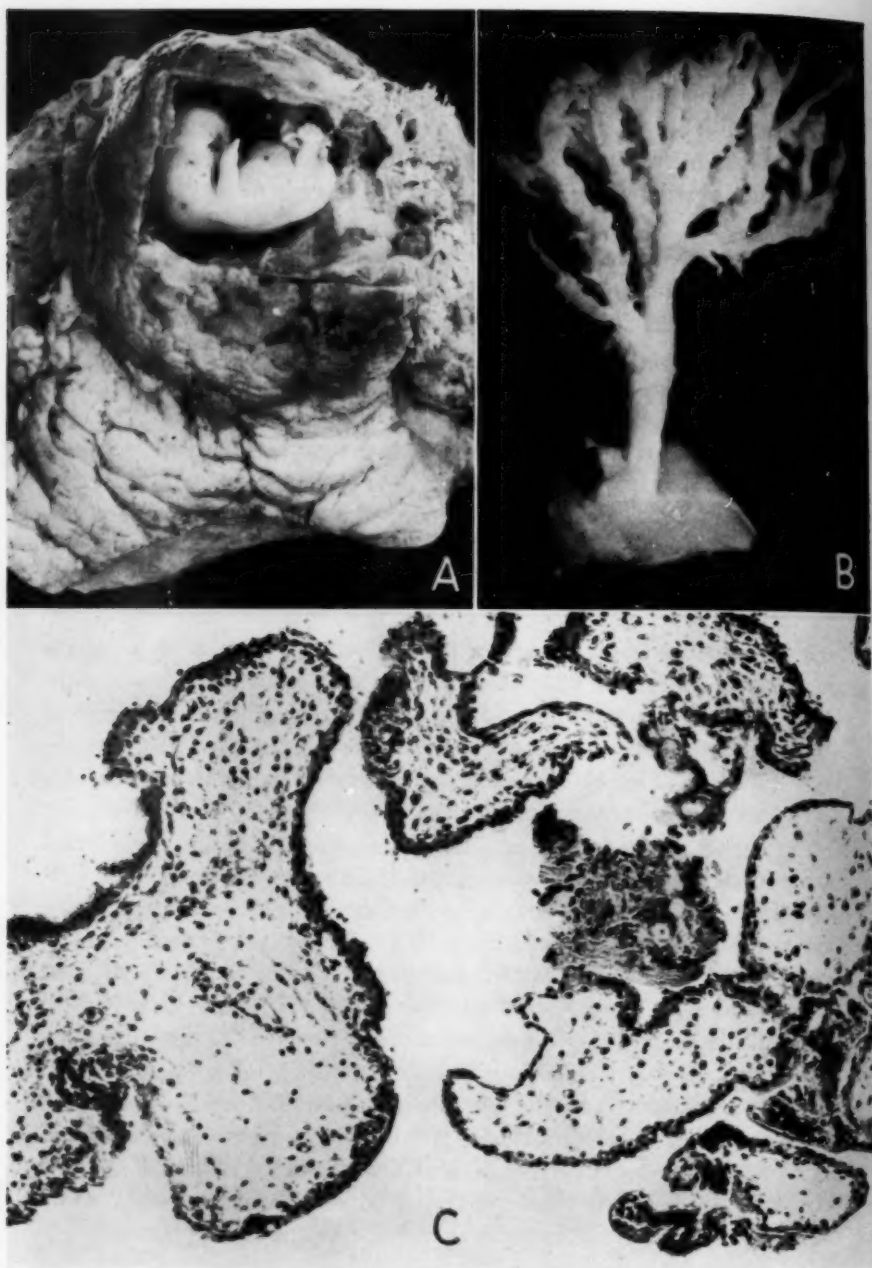


Fig. 1.—*A*, normal embryo and membranes in situ, eight weeks' menstrual age, removed by therapeutic hysterectomy; $\times 1.5$. The decidua vera is below and to the left. *B*, villus from the chorion frondosum of the conceptus shown in *A*; $\times 12$. *C*, section through normal chorionic villi of a normal ten weeks' pregnancy; $\times 140$.



Fig. 2.—Section through villi of a normal nine weeks' pregnancy, taken near the junction of the chorion frondosum and the chorion laeve; $\times 145$. Initial hydatidiform change is evident in the two relatively large and vacuous villi, just above the center of the field, and in two similar villi in the lower right hand corner.

months (the yearly distribution is shown in table 2) 326, or 66.9 per cent, showed early hydatidiform degeneration of the chorionic villi.

There is no significant difference in incidence of hydatidiform degeneration among the various groups of pathologic ova with the exception of group 7. Nor is there any significant difference in incidence of hydatidiform degeneration among intact specimens as contrasted with ruptured specimens of any one group. During this period the most common type of pathologic ovum studied was that of group 3, i.e., a chorion containing an empty amnion. This group constituted 38.4 per cent of all pathologic ova exclusive of group 7, the members of which from a purely embryologic point of view are, or at least started out as, essentially normal ova. Since this variety of abortus (group 3) is the most common single type in all the material from cases of spontaneous

TABLE 2.—Incidence of Hydatid Degeneration in Pathologic Ova Examined in Period from September 1934 to December 1939, Inclusive*

Year	Pathologic Ova	Number with Hydatid Degeneration	Percentage with Hydatid Degeneration
1934-1935.....	51	50	98.0
1936.....	84	66	78.5
1937.....	106	78	73.5
1938.....	105	67	63.8
1939.....	111	65	58.5
Total.....	487	326	66.9

* Exclusive of group 7.

abortion, constituting 18.7 per cent of the total, it is chosen to illustrate the early stages of hydatidiform degeneration.

Figure 3 depicts such a pathologic ovum. It was obtained, intact, sixty-eight days (nine and five-sevenths weeks) after the last menstrual period. The external dimensions of the intact chorionic sac are 3.0 by 2.5 by 1.0 cm. The chorionic villi are well distributed over the surface and average 6 to 7 mm. in length. They are multibranched, possessing four to six dichotomous branches, the majority of which show small grapelike or fusiform swellings up to a millimeter in diameter. The junction of the swollen portion with the remainder of the villus is constricted. These changes are barely visible to the naked eye when the specimen is floated out in water and not visible at all when the specimen is out of water. Under the binocular dissecting microscope, at a magnification of approximately 10 diameters the hydatidiform degeneration is clearly evident (fig. 3 B). Even when viewing such specimens with a binocular dissecting microscope it is necessary to immerse them in fluid. Otherwise the villi will mat together and their outlines become indistinct.

The chorionic sac when incised under the dissecting microscope reveals two intact vesicles, widely separated, each about 10 mm. in

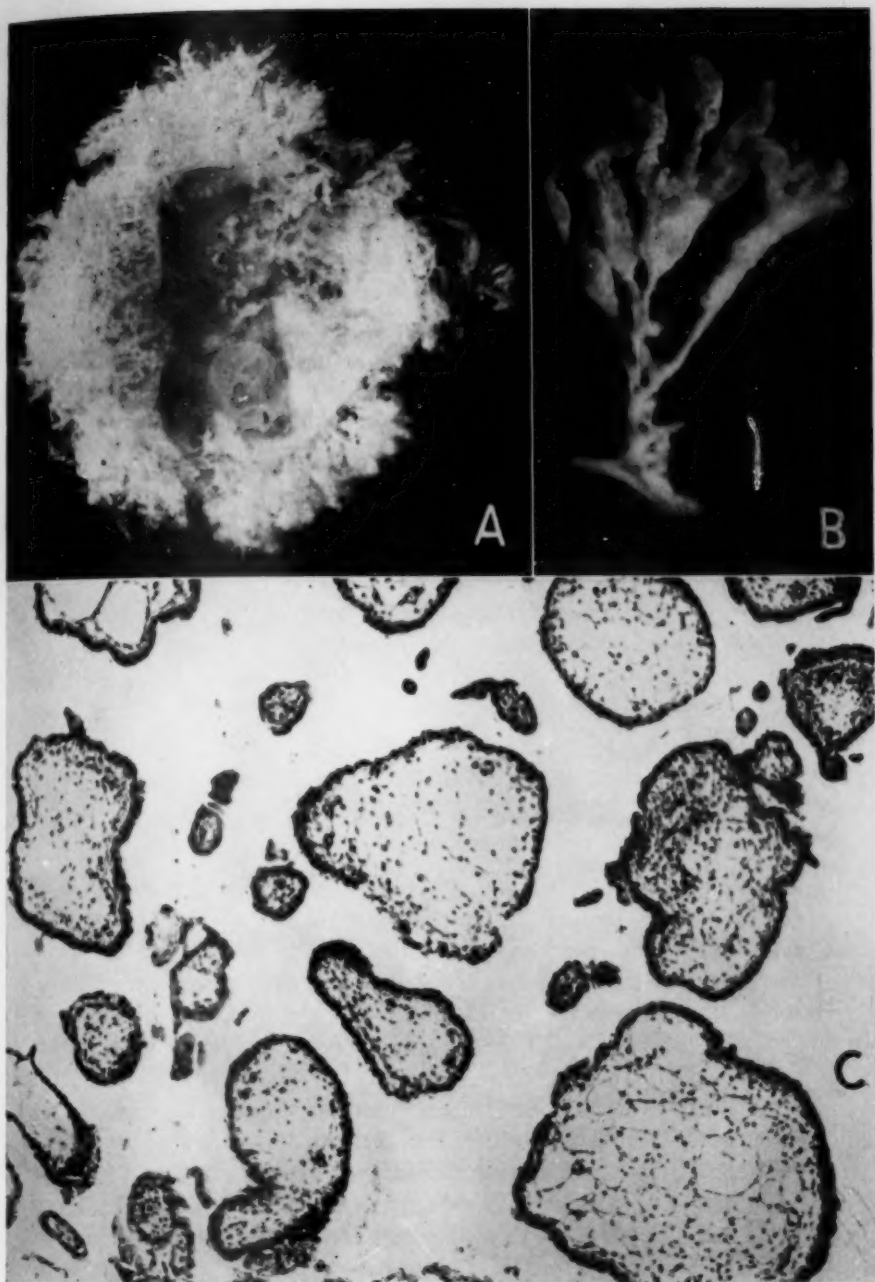


Fig. 3.—*A*, pathologic ovum (group 3-B) aborted at nine weeks' menstrual age; $\times 2.5$. The chorion has been opened, and shows a central cavity containing an empty amnion. *B*, villus from the chorion frondosum of the conceptus shown in *A*; $\times 12$. *C*, section through chorionic villi of a pathologic ovum, nine and five-tenths weeks' menstrual age, showing diffuse initial hydatidiform change; $\times 95$.

diameter. One is slightly vascularized and undoubtedly represents the yolk sac (removed from the specimen prior to photography), whereas the other is thin, pale and translucent, probably representing an abortive attempt at amnion formation (fig. 3 A). There is no evidence of any embryo, in the usual sense of the word, although these two vesicles represent an embryo which became defective and ceased normal development at a time when the normal embryo consists of a bilaminar germ disk, composed of ectoderm and endoderm, contiguous with the equal-sized amnion and yolk sac. This stage of development of the embryo is normally seen at about fifteen days' ovulation age or about twenty-nine to thirty days' menstrual age.

Microscopically these villi often show marked accentuations of the intercellular spaces of the stroma, due to accumulation of fluid. Other villi show slight fibrosis of their stroma. The blood vessels are absent.

TABLE 3.—Incidence of Hydatidiform Degeneration in "Nonpathologic Ova" Examined in Period from September 1934 to December 1939, Inclusive *

	Nonpathologic Ova	Number with Hydatid Degeneration	Percentage with Hydatid Degeneration
1934-1935.....	113	3	2.7
1936.....	109	19	17.4
1937.....	113	14	12.4
1938.....	96	17	17.7
1939.....	109	10	9.2
Total.....	540	63	11.6

* This includes pathologic ovum group 7 (containing macerated but otherwise normal embryos).

The epithelium is all viable and of typical double-layered type, although in places it has undergone benign proliferation. Figure 3 C, taken from a similar specimen, shows essentially the same process but at an earlier stage. Some of the capillaries are still present, while in other villi the endothelium is degenerate or absent. This picture is chosen to show the earliest phase of hydatidiform degeneration that we can recognize microscopically.

(c) Early Hydatidiform Degeneration in Normal or "Nonpathologic" Ova: Of the 540 abortuses that contained a macerated but otherwise normal embryo or a normal nonmacerated embryo or that were from patients who gave a history of having passed an embryo (table 1), there were 63, or 11.6 per cent, that showed early hydatidiform degeneration of their villi. The distribution of these ova by years is shown in table 3.

The average clinical duration of pregnancy in the 78 consecutive cases in which this type of abortus was obtained in 1936 and in which data are available was fifteen and four-tenths weeks. The difference between the mean clinical duration of the pregnancy associated with this type of

abortus and that of the type associated with the pathologic ovum is therefore five and two-tenths weeks, a value statistically significant because it is more than three times as large as its probable error. Pathologically, it is significant that hydatidiform degeneration is nearly six times more frequent in the younger, essentially pathologic ova, which in general have been abnormal from their implantation or became so shortly thereafter, than it is in the embryologically normal ova, which possessed good embryos up to within a few weeks of their abortion. An attempt to explain this difference in incidence of hydatidiform degeneration in the two types of ova will be taken up more in detail later.

The gross and microscopic features of hydatidiform degeneration are usually less marked in this type of abortus than in the truly pathologic type. Fewer villi are involved in the process in "nonpathologic" ova, and the change is much more often picked up microscopically than in the gross. In truly pathologic ova, on the other hand, the degeneration of the villi can often be seen with the naked eye if the specimens are examined critically. Therefore, it seems that this degeneration of the chorionic villi, relatively common in pathologic ova and relatively uncommon in "nonpathologic" ova, can best be explained on the basis of the conditions existent in the ovum at the time the early embryo disappears or stops developing. These various conditions will be discussed later after the remaining stages in the genesis of the hydatidiform mole have been described.

More Advanced Stages of Hydatidiform Degeneration as Seen in Older Ova.—In keeping with the observations of other workers in the field of pathologic embryology, various transitional stages between typical pathologic ova, with frequent early hydatidiform degeneration in the villi, and classic hydatidiform moles have been encountered in this study. Fifteen specimens presenting such stages have been examined grossly and microscopically. They have been designated as transitional moles. The transitional mole may be defined, therefore, as an abortus in which the various portions of the ovum (chorion, villi, amnion and embryo) can still be identified but in which enough villi have sufficient hydatidiform degeneration to make the diagnosis clearly evident from the gross specimen.

(a) Age of Transitional Hydatidiform Moles: The average clinical duration of the pregnancies associated with these moles is sixteen and six-tenths weeks while the average menstrual age of all the typical hydatidiform moles in the present study (74) is seventeen and four-tenths weeks. This includes the aforementioned transitional moles. Excluding the transitional variety, the average age of typical moles is the same, i.e., seventeen and four-tenths weeks. These data are listed in table 4.

The difference between the clinical age of the transitional moles and that of the more typical variety is probably not statistically significant. However, the differences between the three main groups have been shown to be valid by the method of probable error. In the series of 35 moles reported by Das⁴ the mean duration of pregnancy was sixteen and six-tenths weeks.

(b) Description of a Typical Transitional Mole: A typical transitional mole is shown in figure 4. The patient who spontaneously aborted this specimen was nineteen weeks pregnant when the specimen was passed. Grossly, the ovular sac is intact and measures 8 by 3.7 by 2 cm. One portion of the external surface consists of soft, pinkish white placental tissue, the villi of which show early but grossly recognizable hydatidiform swellings. The opposite surface is covered by hemorrhagic decidua capsularis. When opened along the longitudinal axis, vertical to the decidua capsularis, the chorionic sac is found to contain an intact amniotic sac that almost completely fills the chorionic cavity. There is

TABLE 4.—*Clinical Duration of Pregnancies in Which Ova Showed Hydatidiform Degeneration*

Pathologic Ova*	"Nonpathologic" Ova*	Hydatidiform Moles	
		Transitional	Typical
10.2 weeks	15.4 weeks	16.6 weeks	17.4 weeks

* These include abortuses both with and without hydatidiform degeneration of their villi.

a small yolk sac lying between the amnion and the chorionic membrane. The incised amnion is found to contain no trace of an embryo or of a body stalk. The wall of the ovum shows rather massive hemorrhage in portions of the intervillous space. Some villi are typical large cystic structures, occasionally as large as 7 to 8 mm. in diameter (fig. 4A). The majority of the villi, however, show some degree of hydatidiform degeneration.

Microscopically, the stroma has been largely replaced, except at the periphery of the villus, by irregular masses of fine basophilic precipitate (fig. 4B, to the right of the field). The shadows of dilated capillaries are still evident, the endothelium of which is undergoing necrosis. Other villi, such as the one shown toward the left in the same illustration (fig. 4B), possess dense, somewhat fibrosed stroma, from which the capillaries have largely disappeared. The chorionic epithelium has begun to undergo focal hyperplasia of both syncytiotrophoblastic and cytotrophoblastic (Langhans') elements. The syncytial element contains vacuoles, which represent a normal developmental phase of this tissue during the formation of the intervillous space (Hertig and Rock).³¹ The Lang-

31. Hertig, A. T., and Rock, J.: *Anat. Rec. (supp. 2)* **73**:26, 1939.

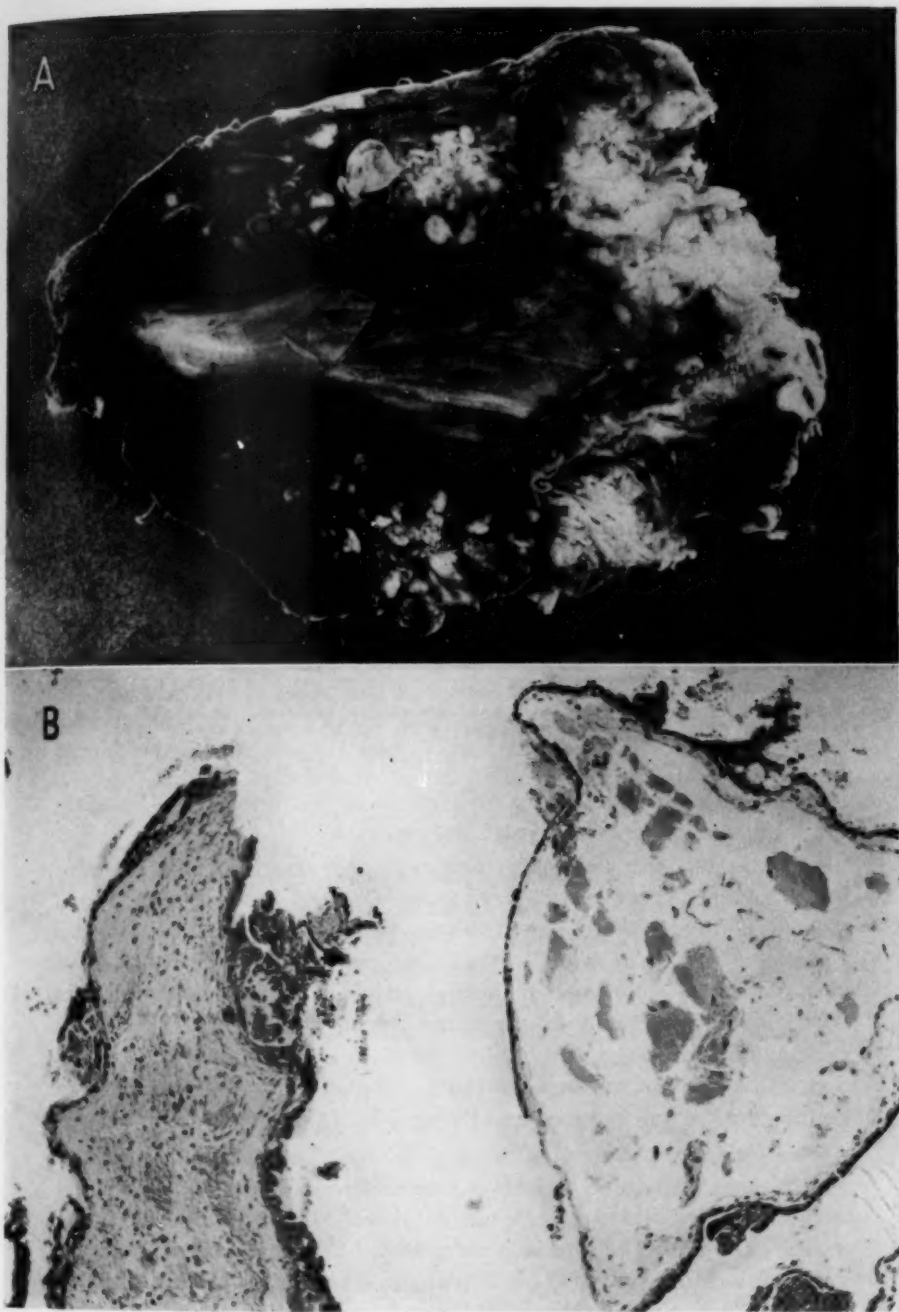


Fig. 4.—*A*, transitional mole; $\times 1.2$. The chorionic cavity is opened to show the empty, folded amnion lying just to the left of the center of the picture. *B*, section through chorionic villi of the transitional mole shown in *A*; $\times 105$.

hans cells form small irregular masses of typical polyhedral cells but show no evidence of malignancy.

There is a certain degree of variation in the appearance of these abortuses which are classified as transitional moles. Four of the specimens possess each a macerated embryo, whereas 11 of them are of the typical pathologic variety with either an empty chorionic cavity or a cavity containing only an amnion within which is a tiny nodular embryo. It is probably not statistically significant that 4, or 26.6 per cent, of these transitional moles contain macerated but otherwise normal fetuses although it is interesting that this incidence is of the same general order of magnitude as the incidence of conceptuses with nonpathologic embryos showing hydatidiform degeneration, namely, 11.6 per cent. Table 5 shows the distribution of these 15 transitional moles with respect to their pathologic type. It is evident, therefore, from even this small group that there exist transitional forms of the hydatidiform mole, the hydatidi-

TABLE 5.—*Distribution of Typical and Transitional Hydatidiform Moles with Respect to Mall's Classification of Pathologic Ova*

Group	1	2	3	4	5	6	7	Total
Transitional moles.....	0	5	5	1	0	0	4	15
Typical moles.....	44	7	3	1	0	0	0	55*

* This total includes only specimens from cases in which the protocols contain definite statements as to the presence or absence of a chorionic membrane. There are 4 of the 59 typical moles about which uncertainty exists on this point, accounting for the apparent discrepancy in the total number.

form degeneration in which is sufficient to be seen grossly and in which the original type of ovum is still demonstrable.

In keeping with the degree and type of hydatidiform degeneration in the younger group of ova, both pathologic and "nonpathologic," considered in the section headed "Early Stage as Seen in Normal and Pathologic Ova" the degree of hydatidiform degeneration in the 4 ova with macerated embryos is neither as striking nor as widespread as in the 11 in which the embryos are either absent or very defective. This is perhaps of significance in regard to the cause of hydatidiform degeneration, although a discussion of the cause of hydatidiform degeneration does not properly belong in a paper on the genesis of this condition.

Typical Hydatidiform Mole.—All of the 59 typical moles in this series (deducting the 15 transitional moles from the total of 74 hydatidiform moles of both varieties) can be classified as true pathologic ova according to Mall's¹ classification (table 5). However, these specimens were not included among the 487 pathologic ova analyzed in the section headed "Early Stage as Seen in Normal and Pathologic Ova" because one of the purposes of this report is to show that typical moles have their origin in this type of pathologic pregnancy.

It is evident from table 5 that in the vast majority of cases (80 per cent) the classic mole consists only of masses of typical hydatidiform villi. These variously sized cystic villi may occur joined together in strings, in groups without reference to their previous villous pattern or even as isolated cysts with the tiny stem detached from the parent mass. Smaller but significant numbers of typical moles are present in the series in each of which a definite chorionic cavity can be demonstrated. Occasionally there is one in which an amnion either with or without a tiny nodular embryonic mass is found. It is our opinion that the few moles of the latter type, 10, occurring as they do in such a relatively large series of true hydatidiform moles, examined with a good deal of care both grossly and microscopically, are of significance in pointing to the type of ovum from which the typical hydatidiform mole is derived. Differently stated, it might be open to question whether the 326 early pathologic ova possessing definite but early hydatidiform degeneration of their chorionic villi and having an average clinical age of ten and two-tenths weeks really are potential hydatidiform moles (which we believe) but which, fortunately for the patient, aborted; or whether the small group of 11 pathologic ova, similar but more obviously the seat of hydatidiform degeneration and possessing an average age of sixteen and six-tenths weeks, are nearer to being typical hydatidiform moles; but there can be no doubt that the 10 typical moles possessing chorionic and sometimes amniotic cavities are really pathologic ova. The fact that the other 44 typical moles did not have empty chorions or amnions, either with or without extremely defective embryos, at the time of examination does not indicate that such structures did not exist at some time in their evolution. The manner of delivery of these specimens is alone sufficient to destroy the relationships of the various portions of the ovum.

In order to demonstrate what a typical hydatidiform mole may show when opportunity is had to examine the whole uterus in situ, the specimen shown in figure 5 is chosen. This specimen was obtained from a primigravida 23 years of age who was sixteen weeks pregnant, in whose case a diagnosis of hydatidiform mole was made at the Providence Lying-in Hospital. Because of severe antepartum bleeding, it was decided to do a supravaginal hysterectomy, which was done twelve hours before the specimen was received in our laboratory. The walls of the cervix had been sewn together immediately after the operation to insure sterility and to keep the mole in its normal relationship to the uterine cavity. The specimen, measuring 16 by 17 by 11 cm., was opened under sterile precautions, in order to obtain molar tissue for tissue culture. This material was sent to Dr. George Gey of the Johns Hopkins Hospital, who reports that the material is growing satisfactorily.

The uterine wall was then incised laterally, and the two walls of the uterus reflected at the fundus. The uterine cavity was filled with typical

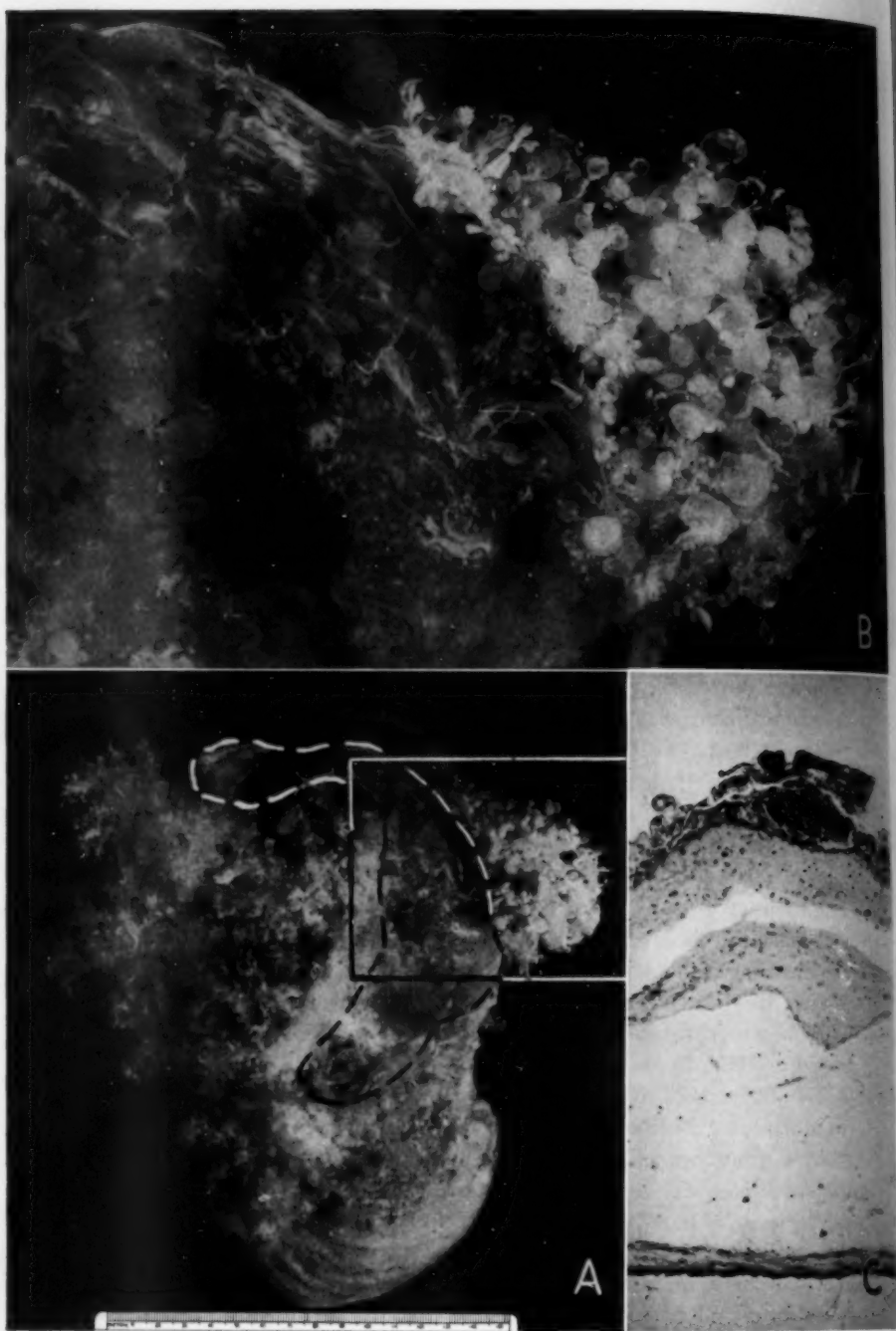


Fig. 5.—*A*, typical hydatidiform mole, sixteen weeks' menstrual age, removed by hysterectomy, opened in situ; $\times 0.4$. The margins of the opened chorionic sac are outlined (broken line). The amnion is closely adherent to the chorion. *B*, detail view of the portion of the mole in the rectangle in *A*; $\times 1.1$. Delicate folds of amnion and chorion appear in the center of the picture. Hydatid villi adjoin the chorionic sac, to right and left. *C*, section through chorion (above) and amnion (below) of the mole illustrated in *A* and *B*; $\times 70$.

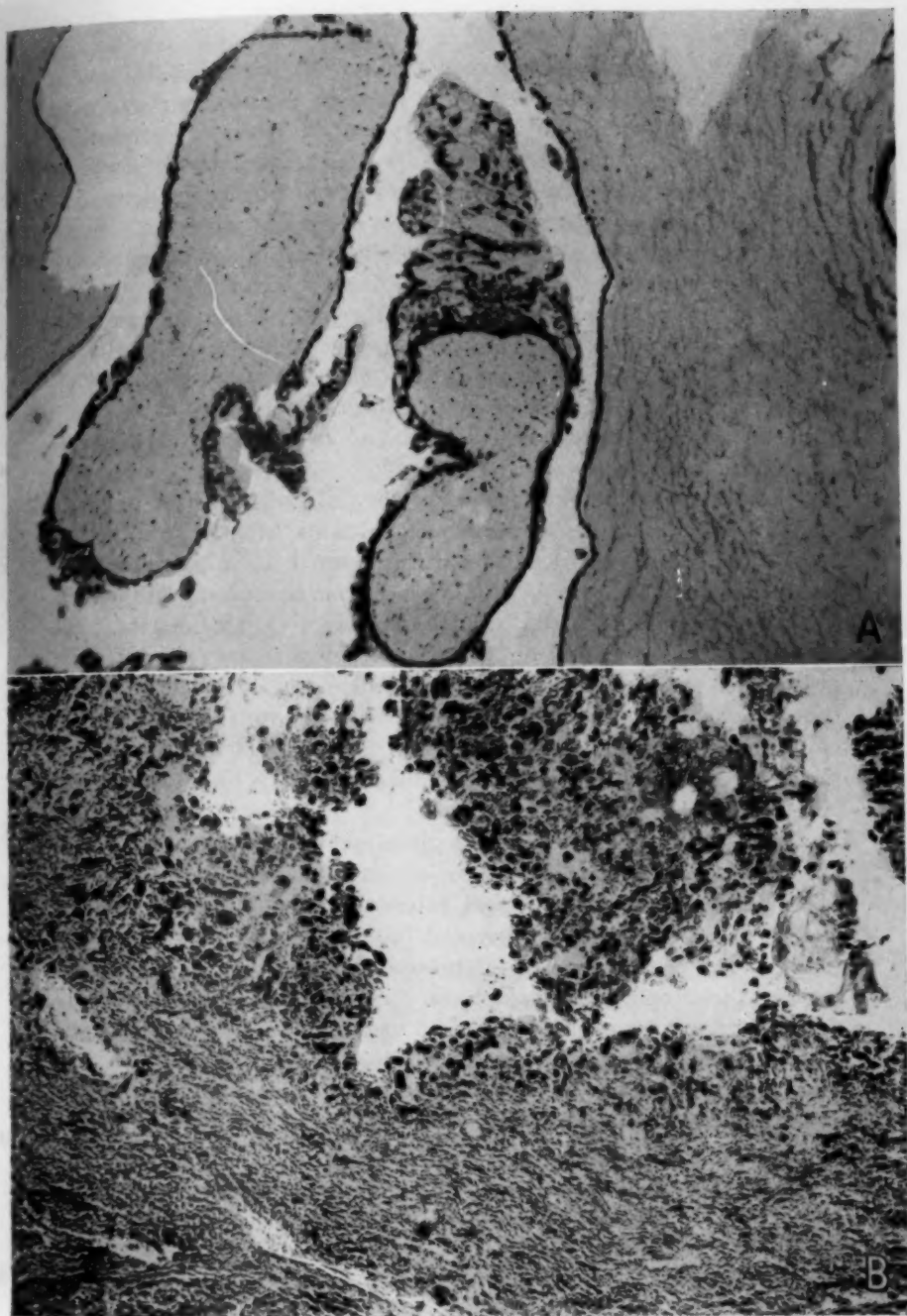


Fig. 6.—Sections from the mole pictured in figure 5; $\times 75$. *A*, hydatidiform chorionic villi. The extensive (benign) proliferation of trophoblastic epithelium just above the center of the field shows a striking resemblance to trophoblast of a normal eleven day ovum. *B*, masses of anaplastic (malignant) trophoblastic epithelium in the upper portion of the field, infiltrating and invading myometrium (lower part of the field).

vesicles of a classic hydatidiform mole, varying from 2 to 12 mm. in diameter. The ovum had implanted itself on the posterior wall. An intact chorion was found, which when incised revealed an intact but empty amnion. The gross relationships of the specimen are shown in figure 5 *A*. A portion of the ovum is shown enlarged in figure 5 *B*. This region shows clearly the membranous nature of the chorion, attached to which are the typical hydatidiform villi of the classic mole. A low power microscopic view of the chorion and the adjacent, somewhat degenerated amnion is shown in figure 5 *C*. The space between the two membranes is due to the persistence of the loose magma reticular that is found normally in the chorionic cavity of the much younger ovum. It is of interest to note that the stroma of the chorionic membrane is also the seat of mild hydatidiform degeneration; i.e., the intercellular spaces are more or less widely separated by fluid. Blood vessels are absent. The epithelium shows the variety of syncytial and Langhans proliferation, together with areas of hyaline degeneration, often seen in the villi from such moles. This tends to indicate that the chorionic membrane itself also undergoes some hydatidiform degeneration along with its villi.

Microscopically, the villi (fig. 6 *A*) show a variable picture. The larger villi are in the form of cysts whose centers contain fine basophilic precipitate. Their walls are composed of loose avascular connective tissue composed of widely separated small immature fibroblasts. Their epithelium, typically double layered, may or may not show any proliferation. Indeed, the epithelium is often atrophic, as it is on the large cystic villi to the right and left in figure 6 *A*.

The smaller villi show diffuse hydatidiform degeneration in their avascular stroma, which is likewise composed of widely spaced mature fibroblasts. The epithelium often proliferates to form peripheral-lying masses of vacuolated syncytium and inner-lying masses of Langhans' cells (cytotrophoblast) such as those seen in figure 6 *A*. All gradations between the atrophic and hyperplastic phases are encountered. In addition there are large irregular detached masses of anaplastic and rapidly growing trophoblast similar to that which has already started to invade the myometrium as seen in figure 6 *B*. The epithelium in these situations is composed of indiscriminately mixed masses of vacuolated primitive syncytiotrophoblast and cytotrophoblast. Many of the cells of the latter possess large irregular hyperchromatic nuclei. Few mitoses are seen.

This specimen might therefore be considered as a chorionepithelioma coexisting with a hydatidiform mole, from which it was derived.

COMMENT

Evidence has been presented from a large series of cases of abortion, in which pregnancy terminated spontaneously, that hydatidiform degeneration is extremely common (66.9 per cent) in pathologic ova in which

the embryos are absent or show evidence of having ceased to develop at a very early stage of their existence. A milder degree of hydatidiform degeneration is much less common (11.65 per cent) in abortuses that possess good, normally developed albeit sometimes macerated embryos. During the eight years in which one of us (A.T.H.) has been connected with the pathologic laboratory of this clinic he has seen only 1 case of hydatid degeneration of the placenta and that of a mild degree, with which there was associated a fetus beyond the legal limit of viability (twenty-eight weeks). It is impossible to state with certainty the total number of pregnancies of which the pathologic ones are represented by the material sent to this laboratory, since outside doctors and institutions contribute specimens from their pathologic pregnancies. It is safe to say, however, that they are in excess of 30,000, since this figure represents the approximate number of deliveries in the ward, private and outpatient departments of the Boston Lying-in Hospital during that time.

It appears, therefore, that the younger and more pathologic the embryos the higher is the incidence of hydatidiform degeneration, while the older and more normal the embryos the lower becomes the incidence of this degeneration. This was simply put by Mall and Meyer,¹ who compared measles with hydatidiform degeneration and concluded that both are very common in the young and very rare in the old.

The true incidence of this process can best be arrived at by considering the incidence of spontaneous abortions. It has been estimated that 11.7 per cent of all pregnancies terminate spontaneously (Taussig³²). This is in close agreement with the conclusions based on the present study, since various members of the staff of the hospital send to this laboratory all the spontaneously aborted material from their private practices. On the basis of their data, approximately 10 per cent is a fair average of the incidence of spontaneous abortion in the locality contributing the material used in this study. Hence we can state with some degree of assurance that early hydatidiform degeneration is present in some form or other in at least 3.2 per cent of all clinically evident pregnancies. This figure is derived from the fact that two thirds of all pathologic ova (groups 1 to 6) show this condition and that these groups, in turn, constitute 47.4 per cent of our material which, in turn, we estimate constitutes a fair representation of spontaneous abortions in general (10 per cent of all pregnancies). If we add the 0.6 per cent hydatidiform degeneration present in the "nonpathologic" ova, or those possessing a normal embryo (52.6 per cent), the figure rises to 3.8 per cent. Furthermore, if we add the transitional moles (which was not done for the purposes of this

32. Taussig, F. J.: *Abortion, Spontaneous and Induced*, St. Louis, C. V. Mosby Company, 1936.

particular study), we find that they constitute 1.44 per cent of the abortuses (of which they certainly are a variety) or 0.14 per cent of pregnancies. This incidence together with that of true moles (1:2,190 pregnancies, or 0.04 per cent—the average of two large American obstetric clinics) would make the total incidence of all forms of hydatidiform degeneration approximately 4 per cent. Since the early stages of hydatidiform degeneration are so common in abortuses, particularly the pathologic ova, one must evaluate the conditions that exist in these ova at the time the degenerative process starts if one is to arrive at conclusions regarding the probable causes.

The typical pathologic ovum showing this degeneration is one in which the embryo is either absent or very defective. For purposes of discussion we may state that the embryo in this group ceases developing during the third and fourth weeks of its growth or during the fifth and sixth weeks in respect to the clinical duration of the pregnancy. This period includes the vital stage when the fetal vascular system is beginning to function (Stieve and Strube³³) and when the vascular "anlagen" in the chorionic villi are beginning to coalesce to form a functional circulation.

At this time the chorion is growing rapidly, the trophoblast is most active and the villi are increasing rapidly in size and assuming their more mature form. The primitive mesoblast, or mesenchyma, that is to form the connective tissue (Hertig²⁵) of the chorion and its villi is very loosely arranged. The normal ovum during its first twenty-one to twenty-two days has grown to an enormous size compared with its minute volume at implantation, mainly by the activity of its trophoblast and the products derived from it, namely, primitive mesenchyma and chorionic vessels (Hertig²⁵). All this growth has gone on in the absence of any functioning fetal vascular system, since the various portions of that system do not join up and function until the embryo has from seven to seventeen somites (Stieve and Strube³³), which corresponds to an ovular age of approximately three weeks. When the embryo is absent, dies early or is very defective (and there is no doubt that some early ova from fertile animals are defective) (Streeter;³⁴ Corner³⁵) the chorion keeps on growing and developing to some extent before the absence or the death of the embryo is biologically perceived by the maternal organism, and the "bad egg" is aborted. It is significant that the average menstrual age of such abortuses is about ten weeks and that they have apparently been defective from the third to the fifth week.

33. Stieve, H., and Strube, I.: *Ztschr. f. mikr.-anat. Forsch.* **32**:107, 1933.

34. Streeter, G. L., in *Cooperation in Research*, Publication 501, Carnegie Institution of Washington, 1938, pp. 397-414.

35. Corner, G. W.: *Contrib. Embryol.* (no. 60) **13**:63, 1921; *Am. J. Anat.* **31**: 523, 1923.

This coincides rather exactly with the average difference, i. e., six weeks, between the stated clinical age of the pregnancy and the anatomic age of the average abortus in which there is a dead but normally developed fetus (Streeter³⁶).

Considering that in the typical pathologic ovum the trophoblastic function is very active at the period when the fetal vascular system would normally begin to function (three weeks ovulation age, five weeks menstrual age), it does not seem remarkable that the normal processes of the trophoblast should go on for a time even though the fetal circulation fails to function because of the lack or the death of the early embryo. Indeed, chorionic function does go on, for in the most defective ova we have seen, namely, those without any amnion or embryo, the size and development of the chorion alone indicate continued development beyond that stage seen in an ovum when the amnion is just beginning to form. This stage normally occurs at eleven to twelve days (Hertig and Rock³¹) and at about the same time for *Macaca mulatta* (Streeter and Heuser³⁷). At this stage the ovum is only a millimeter in diameter and without villi. This is in contrast to the average pathologic ovum, the diameter of which is 2 to 3 cm. and which possesses branched villi, although they are often the seat of hydatidiform degeneration. It seems to us that the loose villous stroma of hydatidiform degeneration is merely an accentuation of the normally loose mesenchyma (or connective tissue) present in the early chorionic villus of the ovum of approximately three weeks' ovulation age or five weeks' menstrual age.

That the early stages of hydatidiform degeneration probably begin at this critical time in the life of a pathologic ovum is deduced from several types of data. On the basis of studies not yet published, one of us (H.W.E.) has shown that when the ratios between the diameter of the swollen hydatidiform villus and the diameter of its constricted portion or stem in a series of chorions showing hydatidiform degeneration and the comparable ratios in a series of normal chorionic villi are plotted against the ages of the respective specimens, the values for the two series form two lines diverging from a point just less than five weeks' menstrual age. From direct observation the earliest gross hydatidiform degeneration seen in this laboratory was observed in an intact abortus of group 2, whose size was that of a normal ovum of five weeks' menstrual age or three weeks' actual ovulation age. There have also been other similar specimens of five to six weeks' menstrual age whose villi showed very early hydatidiform degeneration.

It is agreed by all writers that hydatidiform villi are avascular or are in the process of losing their capillaries. To this we agree. However,

36. Streeter, G. L.: *Contrib. Embryol.* (no. 126) **22**:1, 1930.

37. Streeter, G. L., and Heuser, C. H.: Personal communication to the authors.

we feel that the cause of the disappearance of the vessels is a function of lack, or early death, of an embryo (chorionic vessels develop independently of the embryo [Hertig²⁸]; hence the latter is not necessary for the early stages of vascularization of the chorion, although it is necessary for the functional unity of the fetal circulation), and that the disappearance, degeneration or even necrosis of these vascular "anlagen" is nothing but a variety of disuse atrophy and does not represent a primary abnormality of the chorionic vessels. This is in agreement with the concepts set forth by Hewitt²³ in 1860. We furthermore feel that the often noticed onset of hydatidiform degeneration and the coincidental disappearance of young vessels from villi thus affected are both signs of the same condition, namely, nonfunction of the circulation due to lack, death or maldevelopment of the embryo. In a sense, then, the cause of hydatidiform degeneration appears to be linked up with the lack, or the abnormality, of villous vessels, an expression of which is the accentuation of the normal looseness of the chorionic stroma, due to the accumulation of material passed across, or formed by the activity of the trophoblast. Stated differently, the villus goes on functioning normally except that its imbibition or secretory products have nowhere else to go and thus accumulate in the villous stroma, resulting in the process whose various stages have been traced.

The looseness of the stroma and the activity of the chorionic epithelium at the time when most of the hydatidiform degeneration begins, together with the lack of a functioning fetal circulation, are probably the two factors most responsible for this degeneration. Support for this idea is gained when one considers that with growth and development of the placenta these are the two factors most altered by maturity of the organ. Simple fetal death alone will not result in hydatidiform degeneration in even relatively young specimens. Proof that such cessation of the fetal circulation alone does not cause hydatidiform degeneration once an intact fetal circulation has functioned for a while is shown in the fact that only 42 of 220 (19.2 per cent) abortuses with macerated but otherwise normal embryos showed hydatid degeneration. It will be recalled that the average menstrual age of these specimens was approximately five weeks more than that of the average pathologic ovum. Hence the embryo lived and developed normally for at least five weeks longer than that in even the least pathologic of the abnormal specimens.

When the embryo dies in such a "nonpathologic" specimen, the blood vessels of capillary type disappear, but the larger vessels show organization of the static clots within their lumens. Even though the smaller vessels disappear in these chorions, only occasionally does one see the edema-like hydatidiform swelling of the villi. It will be further recalled

that this hydatidiform degeneration of the stroma is not as marked as in the chorions of pathologic ova and that it is usually picked up on microscopic examination.

The hydatidiform degeneration of the chorion laeve, briefly referred to before, occurs in the portion of the chorion that is normally being excluded from the fetal circulation, hence the villi in that region—still being viable and with a loose stroma and an active trophoblast—could possibly swell up and become typically hydatidiform. That this is a transitory phase is due to the fact that the maternal blood supply soon becomes shut off from that region. Pressure from the overlying decidua capsularis further contributes to the ultimate hyaline degeneration of villi in that region in the full term placental membrane.

There is still another, rarer type of hydatidiform degeneration. Focal areas of immature villi in placentas within the last trimester occasionally show edema of the stroma and fairly active epithelium. The vessels also are somewhat immature. These villous structures give the appearance of new branches of older villi. Should their new vessels fail to join up with the rest of the fetal circulation (new lateral branches of old villi have discontinuous vascular "anlagen" at certain phases in their development) (Hertig²⁵), this immature type of villus might be a potential focus for typical hydatidiform degeneration. Such segmental areas of hydatidiform degeneration do occur rarely in placentas near term (Thaisz²¹).

In younger placentas of the middle trimester, during which occur many of the spontaneous abortions of "nonpathologic" ova, 11.6 per cent of which show early hydatidiform change, this process may well account for the development of the hydatidiform degeneration. The new branches are immature as to epithelium, stroma and blood vessels, even to the discontinuous nature of the latter. Therefore, should the new branches of villi fail to become functionally included in the fetal circulation, the epithelium would probably still continue to function, drawing in or secreting fluid into the underlying stroma.

In the light of the foregoing remarks it would seem appropriate to regard hydatidiform degeneration more as a physiologic than as a purely degenerative process. It must be pointed out that the epithelium and stroma still remain histologically viable, provided the villus remains in contact with a good maternal blood supply. Von Franque, according to Lahm,³⁸ emphasized the secretory activity of the chorionic epithelium in cases of early hydatidiform degeneration. The concept that this process may well be more physiologic than pathologic (always keeping in mind that there is no fetal circulation to carry away the products accumulated by physiologic activity of the villus) is not so heretical when one con-

38. Lahm, W.: *Ber. ü. d. ges. Gynäk. u. Geburtsh.* 4:1, 1924.

siders the various ways in which the typical early viable hydatidiform villus recapitulates the processes in the growth of an early ovum, especially one in the previllous stage. Recently one of the authors (A.T.H.) has been fortunate in finding 2 normal previllous human ova in surgically removed uteri. A detailed description of these will appear shortly in *Contributions to Embryology*. The older of the two (about twelve days) was briefly reported in (1939) (Hertig and Rock³¹). Having become familiar with the microscopic appearance of hydatidiform moles during this study, we were struck by the many points possessed in common by moles and early normal human ova.

The early human ovum is a hollow vesicle, as is the hydatid villus, lined by a loose primitive mesoblast whose inner surface shows a transitory mesothelial-like membrane. Such a membrane was first described for the macaque monkey, by Heuser.³⁹ Occasional hydatid villi show this membrane-like inner surface of the peripheral connective tissue beautifully. The cavity contains a fluid from which fine granules are precipitated by fixation. These are usually basophilic in hydatidiform degeneration and eosinophilic in the early ovum (although the fixatives used are not the same).

The stroma of the early ovum and that of the early hydatid villus are both essentially avascular; the vessels of the former are in the process of formation as discontinuous vacuolated angioblastic masses, while in the latter the discontinuous endothelial capillaries (that possibly have never coalesced and hence remain in their embryonic state) are in the process of disappearing. From a practical standpoint each structure is an enclosed vesicle which is taking in fluid, in all probability as a result of the activity of the trophoblast, and has no internal circulation to carry it away; hence it swells up.

The closest similarity between the early ovum and the cystic hydatidiform villus lies in the epithelium. In the former there is a thick layer of proliferating trophoblast, the outer portion being composed of a prominent layer of vacuolated syncytiotrophoblast and an inner portion of cytotrophoblast. In the hydatid villus the same proliferative process is often seen in the epithelium, although it tends to be focal in type. Figure 6A is an excellent illustration of this irregular but marked epithelial activity. The outer, vacuolated syncytiotrophoblast is clearly evident, and the inner focal area of cytotrophoblastic proliferation lies beneath the syncytium and is continuous with the Langhans layer of the villus. This whole area of epithelial growth rather exactly recapitulates the epithelial activity of the early ovum just before the formation of the villus. Indeed, the mass of cytotrophoblastic proliferation accurately resembles

39. Heuser, C. H.: *Anat. Rec. (supp.)* 52:15, 1932; in *Cooperation in Research, Publication 501*, Carnegie Institution of Washington, 1938, pp. 383-388.

the "anlagen" of an early primitive villus. If such a viable, actively growing embryonic structure as pictured and described should *fail* to go on functioning (provided its maternal blood supply were adequate) it would be more remarkable than the fact that the majority *do* continue to function, swell up, become cystic and are classified as showing hydatidiform degeneration. Whether or not it is justifiable to consider the hydatidiform villus as recapitulating this early phase of ovular development, the reverse is certainly true; to the certain knowledge of one of us, an eminent pathologist in another university made a diagnosis of chorion-epithelioma on curettings containing remnants of the trophoblast from a normal fourteen to fifteen day ovum!

Résumé.—Hydatidiform degeneration of the chorion begins as a diffuse swelling of the stroma and terminates in the formation of a central fluid-filled cavity within the villus. The wall of the cystic villus is composed of viable, essentially avascular connective tissue, surrounded by chorionic epithelium, which varies from a normal or thin, atrophic (in some large cysts) layer of Langhans and syncytial cells to an irregularly thickened, hyperplastic layer composed of masses of syncytiotrophoblast and cytotrophoblast.

It has long been known that early phases of this process are common in a high percentage of spontaneously aborted ova and that transitional stages exist between the common, early form and the classic, uncommon hydatidiform mole of pregnancy. An analysis of 1027 spontaneously aborted ova and 74 hydatidiform moles during the past five years confirms and amplifies the foregoing statement.

Typical early hydatidiform degeneration occurs in 66.9 per cent of the pathologic ova that have been examined, this group in turn constituting 47.4 per cent of all the spontaneously aborted ova in the series. In "nonpathologic" ova, constituting 52.6 per cent of the total series, the incidence of early hydatidiform degeneration is 11.6 per cent. This striking difference in incidence can be accounted for on the basis of essential structural differences in the chorions of these two main groups of aborted ova. The pathologic ova abort on an average at ten and two-tenths weeks and possess either no embryos or only very degenerate ones. The embryo, if one exists in the first place, stops developing at a critical time in the life of the early chorion (about the fifth week of menstrual age). In the absence of any functioning fetal circulation the vascular "anlagen" of the chorion disappear, and hydatidiform degeneration appears in the very loose stroma of the villi, due in all probability to the continued activity of the trophoblast rather than to any intrinsic degeneration of the stroma. The fact that the typical hydatid villus resembles a normal previllous ovum in many respects heightens the suspicion that this process is more of a physiologic one, albeit somewhat modified, than a

degenerative one. "Nonpathologic" ova, possessing normal embryos (although these are often macerated), abort on an average five and two-tenths weeks later than do pathologic ova, and therefore the fetal circulation has existed for at least that many weeks longer than it has in the average pathologic ovum. The villous stroma of such "nonpathologic" chorions is denser, and the epithelium less active—factors militating against the onset of hydatidiform degeneration in such specimens.

Transitional moles, constituting 1.44 per cent of the series, are abortuses averaging sixteen and six-tenths weeks in clinical duration. They are usually typical pathologic ova, although 4 of the 15 specimens had macerated embryos, one of which was beyond the twenty-eighth week and therefore legally viable. The hydatidiform villi are often small, and while not all the villi are involved in the cystic degeneration, there are always some large and cystic enough to justify the gross diagnosis of hydatidiform mole.

Typical hydatidiform mole occurs approximately once in every 2,062 full term pregnancies in this clinic, although mild degrees of hydatidiform degeneration occur in 40 per cent of spontaneously aborted ova or in 4 per cent of all pregnancies. In the present series, gathered from many clinics and individual physicians, the classic molar specimens consist entirely of pathologic ova without any fetuses except in a single instance in which the embryo is represented by a tiny nodular mass of disorganized tissue. The mean clinical age is seventeen and four-tenths weeks. In 10 instances it is possible to prove that the ovum contained no embryo or at most merely a nodular mass of embryonic tissue, although in the remaining 44 cases the specimen consists of only hydatidiform villi without a trace of chorionic cavity. This is interpreted as being due to the disruption of the specimen during delivery, so that the normal relationships of the various structures were lost. A typical hydatidiform mole is, therefore, a true pathologic ovum, without an embryo, with marked hydatidiform degeneration of its villi and frequent disruption of its chorion so that the latter is not apparent at pathologic examination. The resulting specimen therefore appears as a disorganized mass of grapelike bodies. This mature manifestation of hydatidiform degeneration varies in no way, except in degree, from that seen frequently in spontaneously aborted pathologic ova which show all gradations in the evolution of the typical lesion.

SUMMARY

A series of 1,027 spontaneously aborted ova and 74 hydatidiform moles has been studied with respect to the genesis of hydatidiform degeneration. Hydatidiform degeneration to some degree is common, having occurred in 40 per cent of these spontaneously aborted ova, or in

4 per cent of all pregnancies. Typical stages in the evolution of a hydatidiform mole are described. A classic hydatidiform mole is uncommon, occurring only once in 2,062 full term deliveries in this clinic.

Pathologic ova, of ten and two-tenths weeks' mean menstrual age, constituted 47.4 per cent of the total number of spontaneously aborted ova; 66.9 per cent of the pathologic ova showed early hydatidiform degeneration of the chorionic villi. "Nonpathologic" ova, of fifteen and four-tenths weeks' mean menstrual age, constituted 52.6 per cent of the total; 11.6 per cent of such ova showed early hydatidiform degeneration of their villi.

Hydatidiform degeneration of the chorionic villi of pathologic ova begins in all probability at about the fifth week of pregnancy, the time when the fetal circulation should begin to function. The fetal circulation in the chorion of the pathologic ovum fails to function because of extreme defectiveness or absence of the embryo. The vascular "anlagen" disappear coincidentally with the onset of hydatidiform degeneration, both processes being a function of absence or of defectiveness of the circulation.

Hydatidiform degeneration is prone to occur in the villi of early pathologic ova because the stroma of the villi is normally loose and their chorionic epithelium is normally active. Hydatidiform degeneration in the villi of early pathologic ova is an expression of continued physiologic activity of the trophoblast (absorption and/or secretion) with resultant accumulation of intravillous fluid, which cannot be utilized because of the lack of a functioning fetal circulation.

Hydatidiform degeneration is less apt to occur in "nonpathologic" ova, because of a functioning fetal circulation of some weeks' duration, relatively dense stroma and relatively inactive chorionic epithelium.

Transitional moles, of sixteen and six-tenths weeks' mean menstrual age, are usually typical pathologic ova containing no embryos or at most extremely defective ones, although a few contain macerated normal embryos. Typical hydatidiform moles, of seventeen and four-tenths weeks' mean menstrual age, are true pathologic ova possessing no embryos or rarely a very defective one. A typical hydatidiform mole is therefore derived from a true pathologic ovum in which the embryo was either absent or very defective from the beginning and which, for reasons unknown, failed to abort at the usual time. Hence, it constitutes a type of "missed abortion."

VISCERAL LESIONS ASSOCIATED WITH VARICELLA

HARALD N. JOHNSON, M.D.

MONTGOMERY, ALA.

A complete postmortem examination was made on an infant who died during the height of the eruptive stage of varicella. Focal areas of degeneration showing similar pathologic changes were demonstrable in the skin, esophagus, pancreas, liver, renal pelves, ureters, bladder and adrenal glands.

The first histologic description of the varicella lesion of the skin was that by Unna, in 1894.¹ He reported a biopsy of skin from an 8 year old boy, taken during the second day of the eruptive stage of varicella. The principal findings were edema and ballooning of the affected epithelial cells, development of giant cells and formation of confluent cavities at the site of maximum degeneration. No mention was made of any lesion in the dermis or of any specific nuclear abnormalities. The characteristic intranuclear inclusions and the sequence of changes in the corium and epithelium of the skin were described by Tyzzer in 1906.² The material studied was obtained during an epidemic of varicella among prisoners in the Bilibid prison at Manila, P. I. Biopsies were made at various stages of the eruption. Schleussing,³ in 1927, reported visceral lesions in a pair of 3 week old twins who died of varicella. He described focal areas of necrosis in the liver, adrenal glands and spleen. The lesions in the liver and adrenals as shown in his photomicrographs are similar in location and appearance to those to be described in this paper. The literature on varicella contains numerous case reports describing clinical features of the disease not directly related to the cutaneous lesions. Some of the complications mentioned are encephalitis, nephritis, arthritis, synovitis, bursitis, orchitis and blindness. Pathologic confirmation of the specificity of these complications has not been obtained.

REPORT OF A CASE

A 7 month old boy was admitted to the neurologic service of the Children's Hospital, Boston, Oct. 23, 1936, because of hydrocephalus. The maternal and past history were not remarkable. The present illness was of two weeks' duration.

From the Department of Pathology of Harvard Medical School and the Department of Pathology of the Children's Hospital, Boston.

1. Unna, P. G.: *Die Histopathologie der Hautkrankheiten*, Berlin, A. Hirschwald, 1894.

2. Tyzzer, E. E.: *Philippine J. Sc.* **1**:349, 1906.

3. Schleussing, H.: *Verhandl. d. deutsch. path. Gesellsch.* **22**:288, 1927.

During this period the parents noted that the child was less active than normal and that the head was rapidly increasing in size. The general physical examination disclosed no abnormality except the enlargement of the head. Laboratory studies, including estimation of hemoglobin, complete blood cell count, examination of a blood smear, Wassermann and Hinton tests of the blood, urinalysis and examination of the spinal fluid, showed no significant variation from the normal. Combined ventricular and lumbar puncture was done and dye injected into the ventricular system; dye did not appear in the spinal fluid from the lumbar tap, and ventriculograms demonstrated marked enlargement of the lateral ventricles. On October 30 a cerebellar exploration was performed and an attempt made to free adhesions about the foramen magnum. Following the operation there was temporary improvement in the patient's condition, but it was soon followed by progressive debility. On December 9 the patient was exposed to varicella. Three days later 10 cc. of pooled adult human serum was given intramuscularly, and the child was transferred to the isolation ward. Eighteen days after exposure varicella lesions appeared on the abdomen, lower part of the back and buttocks. During the subsequent three days successive crops of lesions appeared on the skin of the scalp, face, trunk and extremities, and the temperature rose from normal to 102 F. Death occurred on the third day after the appearance of the cutaneous eruption.

Necropsy.—The examination was begun two and one-half hours after death. The clinical diagnoses of hydrocephalus and varicella were confirmed. Thrombosis of the superior longitudinal sinus was apparently the immediate cause of death. For the sake of brevity, the entire protocol is not included.

Grossly, there were numerous vesicles, papules and petechiae scattered over the skin. These lesions were most numerous over the scalp and over the lower part of the trunk and thighs, although a moderate number were present over the entire body, including the face. Most of them were sharply demarcated round vesicles, measuring 2 to 5 mm. in diameter, filled with clear yellow fluid and surrounded by a small red areola. There were a moderate number of dull red papules, slightly elevated above the surrounding skin and measuring 2 to 3 mm. in diameter. Dull red petechiae were found in small numbers over the entire skin. A few of the vesicles contained cloudy, opalescent fluid. None of the lesions was pustular, and no flattened or crusted lesions were seen.

The corneas were clouded and showed small superficial ulcerations. Slight circumcorneal hemorrhage was present bilaterally. The fluid in the anterior chamber of each eye was cloudy. The buccal mucous membrane was normal. On the lingual aspect of the gum adjacent to the lower middle incisors there was a shallow ulceration 0.4 cm. in diameter. The mucous membrane of the hard palate presented numerous linear ulcerations, which averaged 3 mm. in length and 1 to 2 mm. in width. These ulcerations had hemorrhagic bases. The tongue and tonsils were not remarkable.

The superficial lymph nodes were not enlarged. The genitalia were of the normal male type. The ankles exhibited moderate pitting edema.

The peritoneal and pleural cavities were not remarkable. The mediastinal structures were normal. The thymus weighed 3 Gm. The pericardial cavity contained a normal amount of fluid, and the lining of the cavity appeared normal. Cultures of blood taken from the right auricle produced no growth. Complete examination of the heart demonstrated no abnormalities. The heart weighed 46 Gm. (normal weight, 38 Gm.). The lungs were normal in appearance and weighed 99 Gm. (normal weight, 100 Gm.). The bronchi were not remarkable. The hilar lymph nodes were of normal size and appearance. The spleen weighed 32 Gm. (normal weight, 20 Gm.). The capsular and cut surfaces were normal.

The mucous membrane of the esophagus showed five small, shallow, ulcerations. These ulcerations were present in the upper, middle and lower portions. They were pale yellow and measured 1 to 3 mm. in diameter. The mucous membrane in general was normal. The stomach, small intestine, appendix and large intestine were normal. The pancreas weighed 14 Gm. It was of the usual size and shape, but the consistency was finely nodular, and on sectioning small firm areas were encountered, although no areas of necrosis were visible. The pancreatic ducts were normal. The liver weighed 256 Gm. (normal weight, 260 Gm.). The liver was not remarkable in consistency or in color. The gallbladder was normal. The right kidney weighed 31 Gm. (normal weight, 31 Gm.). The left kidney weighed 29 Gm. (normal weight, 30 Gm.). The capsular and cut surfaces of the kidneys were not unusual. The renal pelvises were normal. In the middle of the right ureter there was a linear area of red discoloration measuring 2 by 6 mm. The mucous membrane of the ureters otherwise appeared normal. The adrenal glands were not remarkable. Their combined weight was 10 Gm. The bladder was contracted and contained 3 cc. of cloudy, pale yellow fluid. The mucosal surface showed no definite ulcerations, but there were several areas of dull red discoloration measuring 1 to 3 mm. in diameter. The ureteral orifices were normal. The testes were not remarkable. The aorta and inferior cava were normal. The larynx and trachea presented a normal-appearing mucous membrane. The thyroid was normal in size and appearance. The bone marrow was deep red. The costochondral junctions of the ribs were not remarkable.

The brain weighed 1,200 Gm. (normal weight, 750 Gm.). The cerebral convolutions were flattened, and there was no free fluid in the subarachnoid space over the cerebrum. The subarachnoid space over the cerebellum, however, was distended with fluid, with resultant compression of the superior portion of the cerebellum. The ventricular system was generally dilated to a marked degree. The cortex of the frontal lobes measured only 5 mm. in thickness. The foramina of Monro, Luschka and Magendie were very large. The superior longitudinal sinus contained an antemortem thrombus. The middle ear and mastoid process on either side were normal. The pituitary was of average size. The spinal cord in general was not remarkable in appearance, but when it was sectioned the neural canal was found to be dilated. It measured 1 mm. in diameter in the cervical part of the cord and 2.5 mm. in the lumbar portion.

Microscopic Examination.—The sections studied were stained with eosin-methylene blue, hematoxylin and eosin, Wolbach's modification of the Giemsa stain and phosphotungstic acid-hematoxylin. The eosin-methylene blue stain gave the best results in the demonstration of the inclusion bodies, and the cell changes are described as seen in preparations stained by this method.

(a) *Skin:* The changes in the skin were like those described by Tyzzer. The characteristic cytologic changes were best illustrated in the early papule stage of the cutaneous lesion. In the smallest of these the epithelium was slightly elevated because of swelling of the epithelial cells and edema and vasocongestion of the underlying dermis. The endothelial cells of the superficial capillaries were swollen, and the connective tissue was edematous, as shown by fragmentation and separation of the collagen fibrils. The basement membrane of the stratum germinativum was indistinct, and the cells of the germinal layer were often separated by cleftlike spaces. The basal margin of these cells was indistinct, and the protoplasmic bridges could not be identified. Vacuoles were often present between the cells. Mitotic figures were slightly more frequent in the germinal layer of this type of

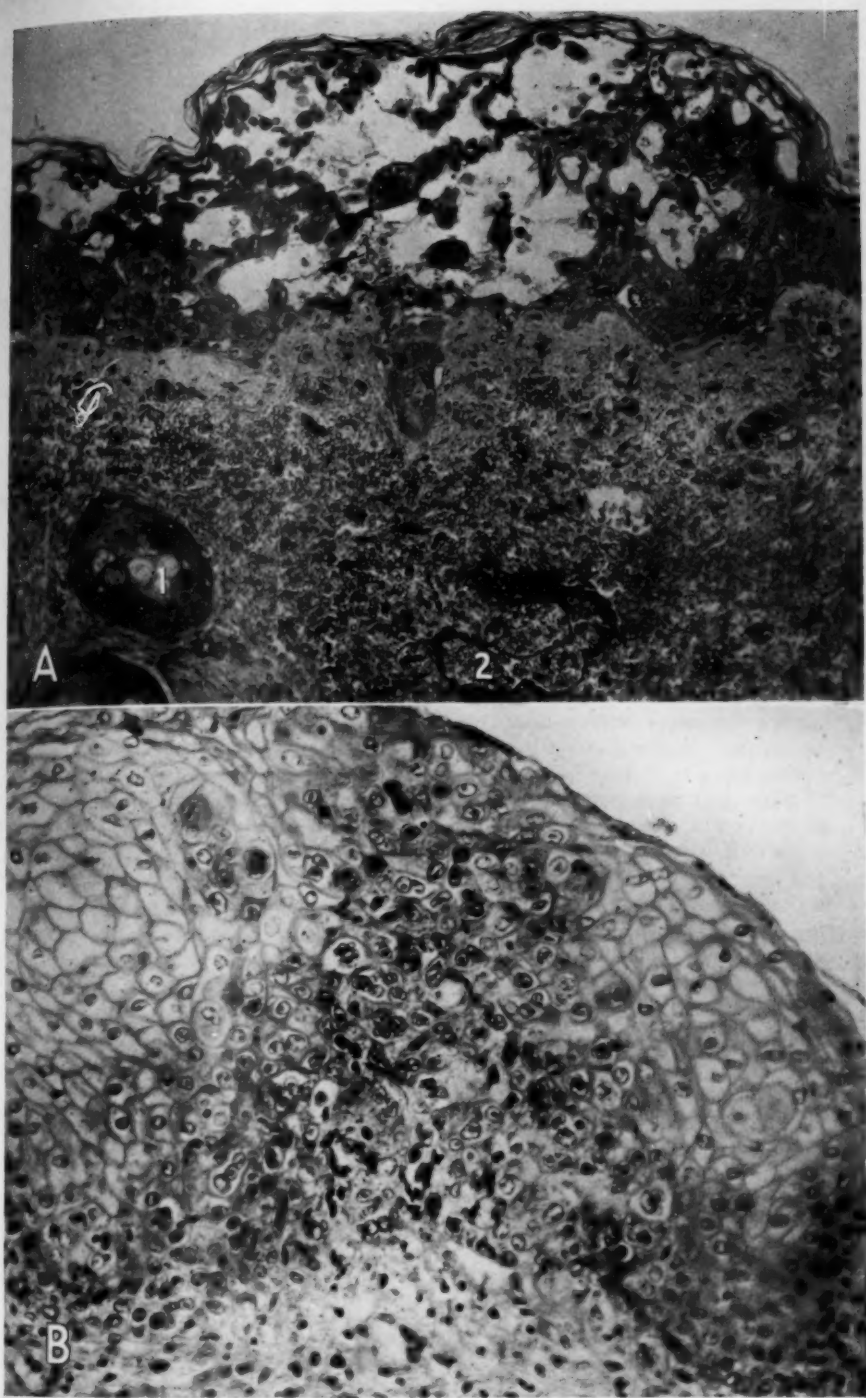


Fig. 1.—*A*, photomicrograph of the skin showing a small varicella vesicle. This illustrates the typical location of the fluid aggregates in the epidermis. At 1 is shown a portion of a hair follicle. Note the prominent intranuclear inclusion bodies in the epithelial cells. At 2 the endothelial cells of a blood vessel have been destroyed, with subsequent perivascular hemorrhage. There are numerous polymorphonuclear and mononuclear cells about this vessel, but the epidermis is free from inflammatory cellular infiltration. Eosin-methylene blue. Approximate magnification, $\times 190$.

B, photomicrograph of a microscopic area of degeneration in the esophageal mucosa. Eosin-methylene blue. Approximate magnification, $\times 280$.

lesion than in the surrounding normal epithelium. In the stratum granulosum there were a few cells undergoing acute degeneration of a nonspecific nature, characterized by nuclear pyknosis, fragmentation and general loss of cell detail. The majority of the affected cells, however, were swollen, and it was these that showed the specific nuclear changes. Occasional giant cells were found in the center of these small foci of epithelial degeneration.

The cytoplasm of most of the affected epithelial cells was increased in volume with concomitant rarefaction and vacuolation. Some of the cells attained enormous dimensions. A similar observation led Unna to call the change "ballooning degeneration." A few of the cells were only slightly enlarged, but these also showed a variable degree of vacuolation of the cytoplasm. The most marked rarefaction of the cytoplasm occurred about the nucleus, leaving an accumulation of the basophilic staining granular coagulum along the cell membrane. In occasional instances the cytoplasm contained aggregates of dark blue granules. A very few of the cells contained intracytoplasmic inclusion bodies staining blue to copper red and measuring 1 to 3 microns in diameter. These were usually well circumscribed. Sometimes a single cell contained one to three such bodies.

The most interesting changes were to be found in the nuclei. There was a slight to moderate increase in size, comparable to the ballooning of the cytoplasm. The chromatin net and nucleoli were aggregated along the nuclear membrane, leaving the major portion of the nucleus clear and vesicular in appearance. In the central portion there was usually a mass of acidophilic staining material, measuring 2 to 5 microns in diameter. These masses averaged 3.3 microns in diameter and were characteristically round, sharply demarcated and bright pink to copper red. The developing inclusion was an indistinct mass of finely granular acidophilic material in the center of the nucleus. The margins were feathery, and the shape approximated that of the nucleus. The more mature inclusion was sharply demarcated, deep pink and had a refractile, hyalin-like appearance. A few inclusions presented a coarsely granular appearance. The central localization of the varicella inclusion, surrounded by a clear space and, in turn, by a deeply staining nuclear membrane has led previous observers to coin the term "bird's eye inclusion." The nuclear membrane sometimes appeared very thick, owing to the granular coagulum of nuclear chromatin adjacent to it.

As previously mentioned, there were a few giant cells in these small epithelial lesions. These often had 5 to 8 nuclei, closely approximated and surrounded by an indefinite membrane. The nuclei of these giant cells uniformly contained inclusion bodies such as those previously described. It was often possible to find two or three closely approximated nuclei with recognizable, though poorly defined, individual cell membranes. It was of interest to note that in comparing the number of nuclei in an area of epidermal degeneration with that of an analogous area of normal epidermis the total was approximately the same. The nuclear membrane appeared to be destroyed much later than the cell membrane. There were occasionally as many as ten nuclei in one giant cell.

There was a variable number of vacuoles between the epithelial cells of the stratum granulosum, and sometimes there was a red coagulum between the cells. The stratum corneum was not affected.

The endothelial cells of the capillaries showed a general increase in volume. In rare instances there were intracytoplasmic inclusion bodies such as those described in the epithelial cells. The nuclei often contained large acidophilic inclusion bodies similar to those of the epithelial cells. No giant cells were to be found in the dermis.

The lymphatics of the superficial dermis were dilated, and the cells lining these structures showed cell changes and intranuclear inclusion bodies like those in the endothelial cells of the blood vessels.

The occurrence of edema and of cytoplasmic and nuclear changes, like those just described, in the superficial dermis, where the epithelial cells were normal or only slightly affected, suggests that the vascular may precede the epithelial lesion.

The sequence of changes from the small papule to the vesicle were: progression of the epithelial degeneration, coalescence of the intercellular vacuoles and spread of fluid from the edematous corium. In some instances the fluid first appeared at the junction of the corium and epidermis, but for the most part it collected first in the stratum granulosum. The large vesicle was the result of the union of several centers of epidermal degeneration. The septums formed by the approximation of the fluid-filled cavities soon ruptured, leaving a single large cavity. The vesicle fluid contained masses of degenerated cells which had lost all structural detail except for occasional persistence of a nucleus. The degenerated cells forming the wall of the vesicle had an acidophilic staining reaction. There were numerous giant cells about these large vesicles, and those nearest the lining of the cavity consisted of closely approximated nuclei surrounded by an acidophilic hyalin-like material. Small clumps of copper-red refractile material were present between the cells surrounding the vesicle, and similar masses were found in the fluid of the vesicle. There was rarely any inflammatory cellular infiltration of the areas of epidermal degeneration during the early vesicle stage. No bacteria were seen in the unruptured vesicles.

The vascular changes in the dermis described in the minimal lesion were succeeded by complete necrosis of the vascular endothelial cells, thrombosis and hemorrhage into the dermis. In a few instances there was hemorrhage into the dermis where the epithelium showed only slight degenerative change. At the sites of thrombosis and hemorrhage the connective tissue showed marked degeneration, edema and a variable infiltration of mononuclear and polymorphonuclear cells.

The epithelial cells of the hair follicles and sebaceous glands were affected in a manner similar to that of the general epithelium. The sweat glands were also affected, but the epithelial degeneration was almost entirely limited to the ductal portion, and the epithelial cells of the coil gland showed no specific change.

The arteries, veins and nerves of the stratum reticularis were normal in appearance.

(b) Esophagus: There were numerous microscopic areas of epithelial degeneration in the esophageal mucosa. In the sequence of changes that constituted their development and in the pathologic aspects of the cellular degeneration these lesions were similar to those of the skin. The early lesion was characterized by degeneration of the basal epithelial cells and of the endothelial cells of the adjacent blood vessels. The affected cells showed swelling with rarefaction and vacuolation of the cytoplasm. The nuclei contained acidophilic staining inclusion bodies, and the chromatin material was approximated to the nuclear membrane. The lesions differed from those of the skin in that there were only occasional giant cells, and these contained fewer nuclei than those seen in the skin. The intranuclear inclusion bodies were prominent, and intracytoplasmic inclusion bodies were more uniform and numerous than those of the skin. The intracytoplasmic inclusion bodies were pink to copper red and measured 1 to 2 microns in diameter. They were round and well circumscribed. The ballooning of epithelial cells was marked.

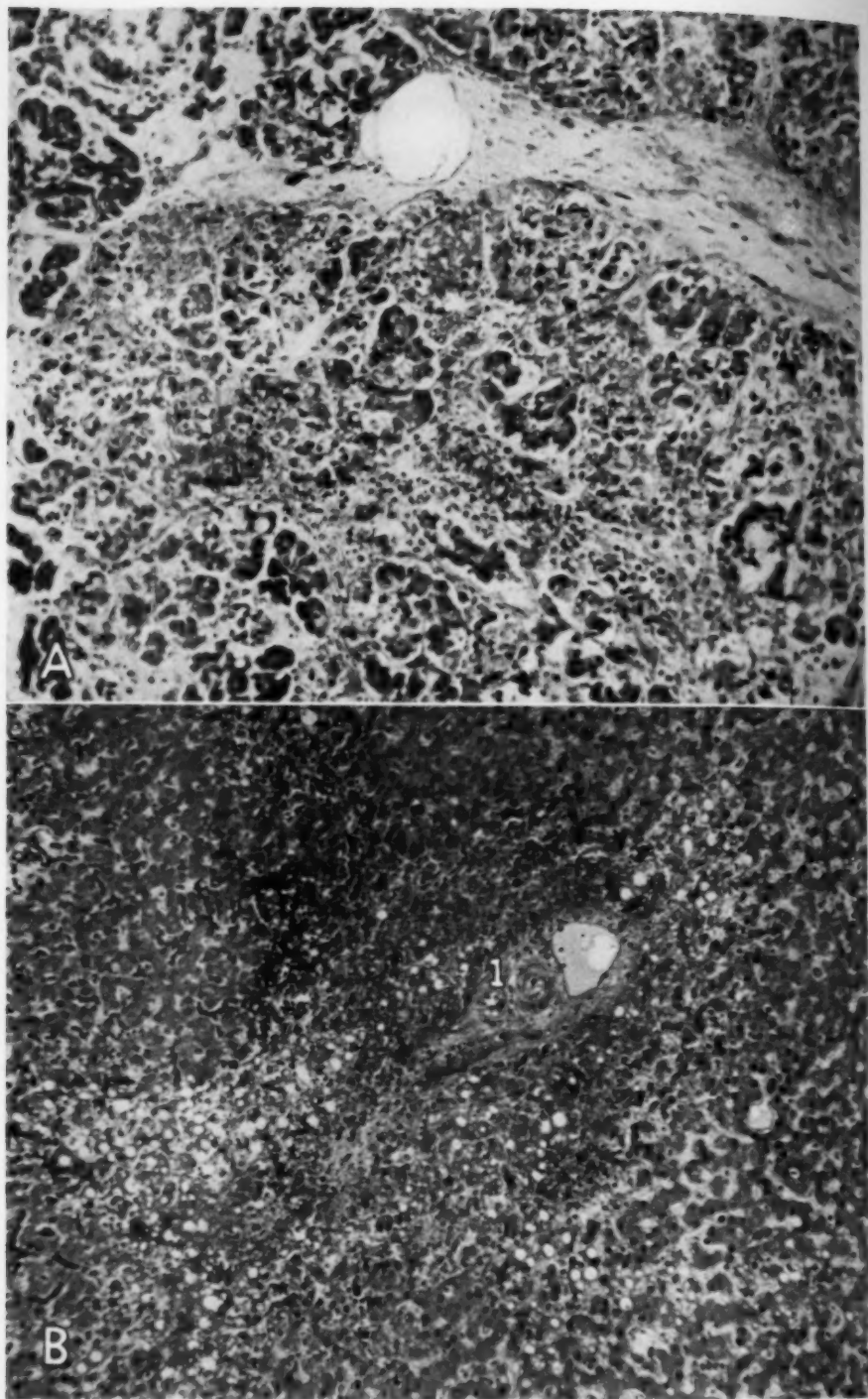


Fig. 2.—*A*, photomicrograph of an area of parenchymal degeneration in the pancreas. Many of the acini are completely destroyed, and the connective tissue stroma is infiltrated by polymorphonuclear and mononuclear cells. Eosin-methylene blue. Magnification, $\times 130$.

B, photomicrograph of the liver illustrating the periportal localization of the cord cell degeneration. At 1 is a bile duct. Eosin-methylene blue. Magnification, $\times 130$.

In the larger esophageal lesions the basal layer of epithelial cells had separated from the submucosa, leaving a space similar to the early vesicle stage noted in the skin. In many instances the entire mucosa had disappeared, leaving an ulcerated, edematous and hemorrhagic base, infiltrated to a variable degree by mononuclear and polymorphonuclear cells. The blood vessels in this sort of lesion were often necrotic and contained fibrin thrombi. The glands of the submucosa were not affected, and the muscularis, nerves and larger blood vessels were normal.

(c) *Pancreas*: There were many focal areas of parenchymal degeneration in the pancreas. Most of these covered only an area of several acini, but sometimes an entire lobule was involved. In the necrotic zones the capillaries were congested, and the connective tissue was edematous, fragmented and infiltrated by mononuclear and polymorphonuclear cells. The nuclei of the capillary endothelial cells contained inclusion bodies like those described. When an entire lobule was affected, there was hemorrhage into the connective tissue.

The individual acini showed two types of degenerative change. In some instances there was acute degeneration with nuclear pyknosis and general loss of cell detail, but the majority of the affected cells showed ballooning and intranuclear inclusion bodies such as were described in the skin.

The epithelium of the pancreatic ducts was normal. The islands of Langerhans were affected only where they lay in or adjacent to areas of acinar degeneration. The major nerves and blood vessels were normal.

(d) *Liver*: The general structure of the liver was well preserved except for occasional small focal areas of necrosis of liver cord cells. These lesions were characteristically periportal and seldom extended more than one third of the way to the central vein. The necrotic zones were sharply demarcated, and the major portion of the affected cord cells were shrunken and stained deep pink. The cord cells along the margin of the necrotic areas were markedly vacuolated, and the nuclei showed margination of the chromatin, but no intranuclear inclusion bodies were demonstrable in these cells.

The terminal bile ducts seemed to be the original foci of the degenerative process. The bile duct epithelial cells in the necrotic zones were for the most part destroyed, but in a few instances swollen epithelial cells were to be found which showed cytoplasmic and nuclear rarefaction and well defined intranuclear inclusion bodies. The adjacent blood vessels were congested, and the endothelial cells were swollen, but no intranuclear inclusion bodies were demonstrable in these vessels. The periportal connective tissue was edematous and necrotic. There was no inflammatory cellular infiltration.

The cord cells about the central veins were well preserved. The Kupffer cells were normal, and the sinusoids were not remarkable. The larger bile ducts and blood vessels were normal. The gallbladder mucosa was not affected.

(e) *Kidneys*: There were occasional foci of epithelial degeneration in the renal pelves. These were to be found in the angles of the calices. Most of the affected epithelial cells were swollen and contained intranuclear inclusion bodies. Some of the cells were shrunken and fragmented. The underlying connective tissue was edematous, and the blood vessels were congested. A few mononuclear and polymorphonuclear cells were to be found about these vessels. The endothelial cells of the capillaries were swollen; otherwise they were not remarkable.

The glomeruli were normal. A few of the collecting tubules appeared to be undergoing acute degeneration of a nonspecific type, characterized by fragmenta-

EXPLANATION OF FIGURE 3

A, photomicrograph of an area of epithelial degeneration in the renal pelvis. At 1 is the nucleus of an epithelial cell, with peripheral dispersion of the nuclear chromatin and a central inclusion body. At 2 is an intracytoplasmic inclusion body. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

B, photomicrograph of an area of epithelial degeneration in one of the ureters. At 1 is the nucleus of an epithelial cell, containing a well defined inclusion body; at 2, a capillary; at 3, a polymorphonuclear cell in the submucosa. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

C, photomicrograph of the dermis of the skin adjacent to a developing vesicle. At 1 is a histiocyte containing an intranuclear inclusion body; at 2, a capillary endothelial cell containing an intranuclear inclusion body; at 3, a dilated lymphatic. The connective tissue stroma is edematous and contains a few red blood cells. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

D, photomicrograph of a capillary in the submucosa of the bladder. At 1 is an endothelial cell within the lumen; at 2, an intranuclear inclusion body. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

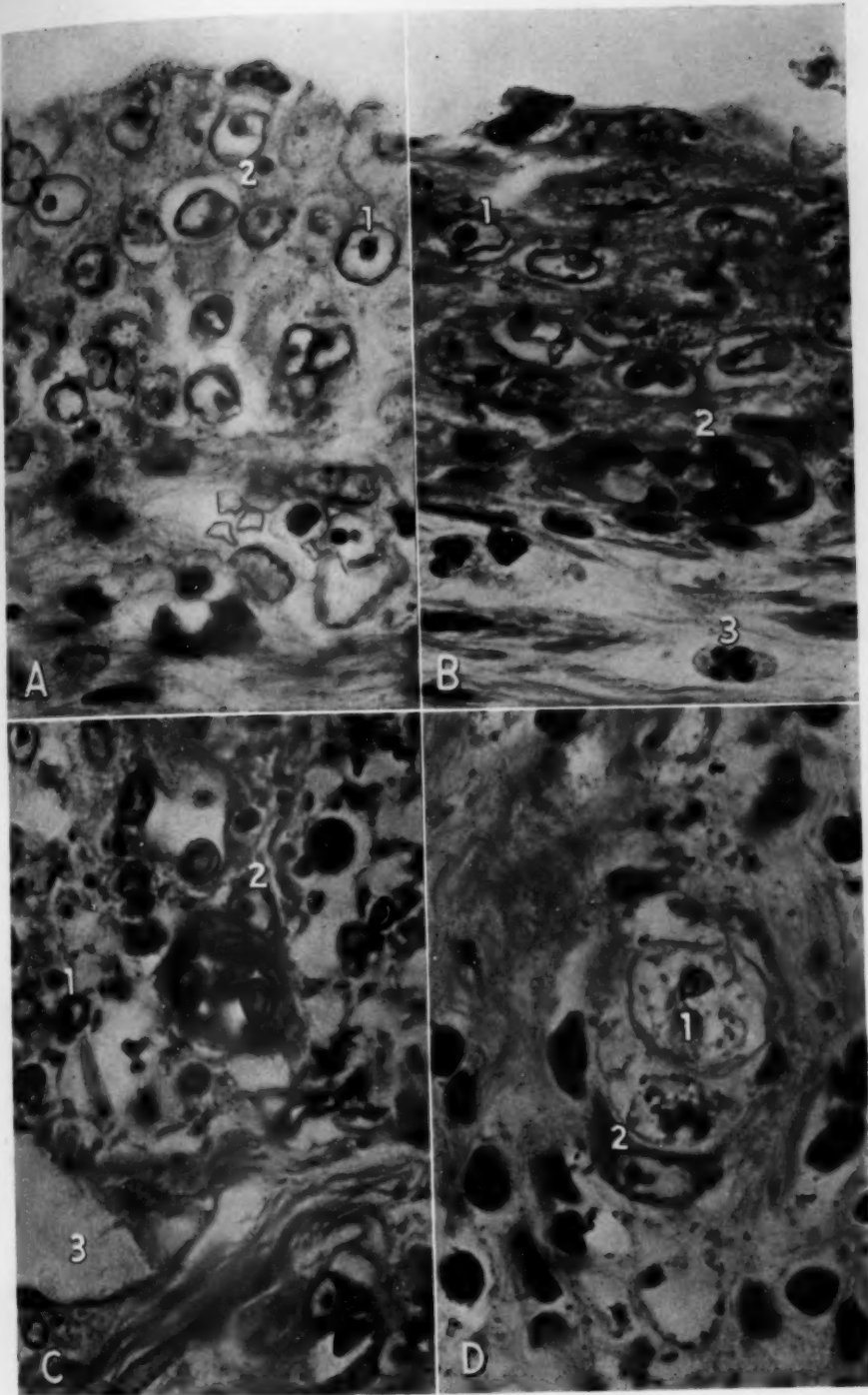


Figure 3

EXPLANATION OF FIGURE 4

A, photomicrograph of the basal epithelial cells in one of the small cutaneous papules. At 1 is a giant cell. Note the clear nucleoplasm, the peripheral dispersion of the nuclear chromatin and the central inclusion body. At 2 is a histiocyte containing an intranuclear inclusion body. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

B, photomicrograph of the bile duct shown in figure 2 *B*. At 1 is an arteriole; at 2, an epithelial cell of a bile duct, containing an intranuclear inclusion body. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

C, photomicrograph of the adrenal cortex adjacent to a zone of medullary necrosis. At 1 note the vacuolation of the cytoplasm of a cell. At 2 is the nucleus of a cord cell containing an inclusion body. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

D, photomicrograph of the adrenal medulla. At 1 is a cell which shows non-specific degeneration. At 2 is a nucleus containing an inclusion body; at 3, a congested capillary. Eosin-methylene blue. Oil immersion; magnification, $\times 1,000$.

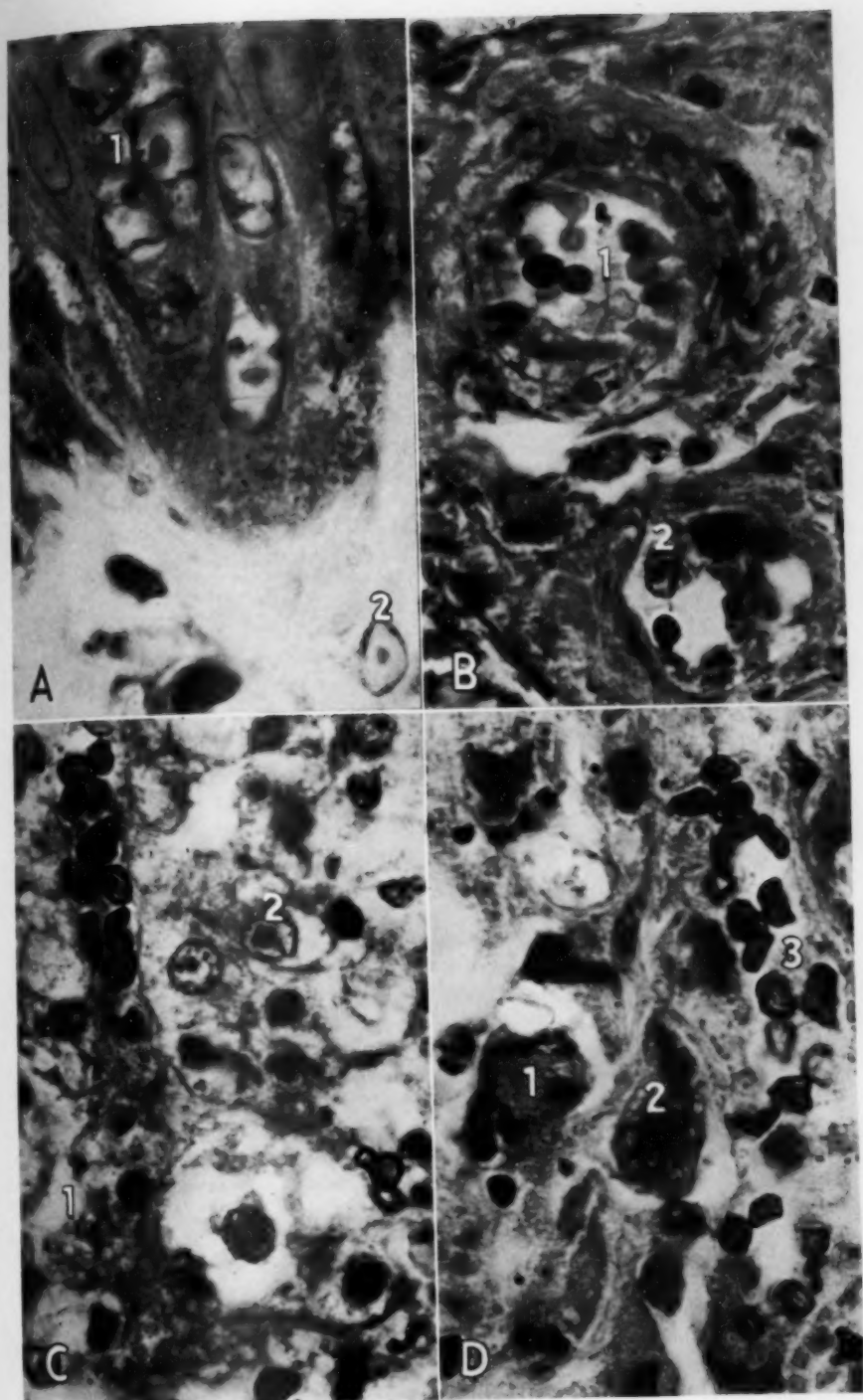


Figure 4

tion and general loss of cell detail. The parenchyma was otherwise normal. The blood vessels in general were not remarkable.

(f) Ureters: A section of ureter taken from the area of red discoloration noted at the time of the gross examination showed a focal area of marked necrosis of the epithelium and underlying connective tissue. The epithelial cells were fragmented and had lost all cell detail. The submucosa was edematous, hemorrhagic and lightly infiltrated by mononuclear and polymorphonuclear cells. The histiocytes and the endothelial cells of the capillaries showed cytoplasmic and nuclear changes like those described in the dermis of the skin.

In other sections of the ureters there were less severe epithelial lesions, and here the cells were swollen and the nuclei contained prominent intranuclear inclusion bodies. The submucosa in these areas was edematous and contained occasional mononuclear and polymorphonuclear cells. The capillaries were affected in a manner like that noted in the large area of degeneration previously described.

(g) Bladder: The epithelium of the bladder was in general well preserved. There were, however, focal areas where the epithelium was partially or completely destroyed and the underlying submucosa was edematous and hemorrhagic and contained numerous histiocytes and occasional mononuclear and polymorphonuclear cells. The degenerating epithelial cells often contained intracytoplasmic inclusion bodies, but no intranuclear inclusion bodies were demonstrable. The intracytoplasmic inclusion bodies measured 1 to 2 microns in diameter and were copper brown.

The histiocytes and vascular endothelial cells in many instances contained intranuclear inclusion bodies like those described elsewhere, and occasionally a capillary was surrounded by a collar of histiocytes showing such nuclear changes.

(h) Adrenal Glands: The capsule and superficial adrenal cortex were normal. There were, however, focal areas of medullary necrosis. Here the normal cell detail was destroyed, the blood vessels were congested, and the medullary cells were shrunken and distorted. The nuclei were fragmented or pyknotic except for occasional cells which had large vesicular nuclei and contained large intranuclear inclusion bodies. These areas of necrosis were sharply defined but did extend into the adjacent adrenal cortex. Here the cord cells of the cortex were markedly swollen, the cytoplasm was reticulated and vacuolated, and the nuclei showed margination of chromatin and nuclear material and contained large intranuclear inclusion bodies. A very few mononuclear and polymorphonuclear cells were to be found in the affected areas. The capillaries, though markedly congested, showed no specific cellular changes. The major blood vessels and nerves were normal.

(i) Brain and Cord: The histologic study of the brain and spinal cord was complicated by the presence of hydrocephalus and thrombosis of the superior sagittal sinus. Numerous sections were taken of the central nervous system, including representative sections of the cortex with adjacent white matter and ependyma and of the choroid plexus, basal ganglions, hippocampus, pons, pineal gland, pituitary, medulla and cerebellum, as well as numerous sections of the spinal cord. In no instance were there any cell changes such as those noted in the epithelial cells of the skin, and no intranuclear inclusion bodies were seen. A moderate number of the cortical pyramidal cells showed acute degeneration, characterized by loss of cell detail, shrinkage of the entire cell and pyknosis of the nucleus. There were also other changes in the cortex and white matter, such as edema and fragmentation of the glial tissue and subependymal degeneration of the white matter with associated infiltration of macrophages (*Gitterzellen*).

The edema was most marked about the Virchow-Robin spaces. The thrombus in the superior sagittal sinus showed early connective tissue organization and appeared to be of several days' duration. The choroid plexus contained a few areas of necrosis, and these were infiltrated by mononuclear and polymorphonuclear cells. Some of the mononuclear cells contained amber-colored pigment. There were no areas of hemorrhage into the brain substance. The pons, basal ganglions, pineal gland, cerebellum and pituitary were normal in appearance. An occasional ganglion cell in the medulla was condensed and pyknotic, but in general the cells were well preserved. The pyramidal area showed degenerative changes in the anterior motor tracts, i. e., swelling and vacuolation of the myelin sheaths and swelling of the axons.

The cord, not being involved by the thrombosis of the dural sinus, should have been easier to study, but here the neural canal was dilated and the general structure of the cord distorted. The cells of the anterior horns were in general well preserved, but here again a few of the cells showed acute degeneration such as was described in the medulla. As in the brain, no inclusion bodies were seen. In the dorsal nerve roots and also in the cord certain fiber tracts showed vacuolation and swelling of the myelin sheaths. Some of the sections contained portions of the dorsal root ganglions, and here, though most of the nerve cells were well preserved, there were occasional cells that showed acute degeneration similar to that described in the pyramidal cells.

(j) General Histologic Observations: The sections of the heart and lung showed no significant abnormalities. The spleen was normal except for lymphoid depletion and slight degenerative changes in the malpighian bodies. The stomach and the small and large intestine had a well preserved mucosa, and no focal areas of epithelial degeneration were observed. The submucosal nerve plexuses, muscularis, nerves and blood vessels were not remarkable. The cord cells and interstitial cells of the testes were normal. The tracheal mucosa was not remarkable. The lymphoid tissue of the thymus was depleted. There were numerous Hassall's corpuscles and a few DuBois sequestrums. The thyroid, parathyroid, tongue, aorta and diaphragm were normal. The epithelium over the tonsils appeared normal. The parenchyma showed lymphoid depletion and inactive germinal centers. There was an area of necrosis in the peritonsillar connective tissue. This was heavily infiltrated by polymorphonuclear cells, and the connective tissue was edematous and necrotic. No bacteria were seen, and no specific cell changes were noted. A portion of the sublingual salivary gland was included, and the acinar and ductal epithelium was normal. The submaxillary gland was normal except for the cells of a few of the acini, which presented a slight amount of acute degenerative change. No nuclear changes such as were described elsewhere were noted. The ducts were normal. Numerous sections of lymph nodes showed no unusual pathologic condition. There was general lymphoid depletion, and the germinal centers were inactive. The bone marrow of the rib and vertebrae was normal. A section of the middle ear and mastoid process showed nothing abnormal.

COMMENT

In some of the virus diseases the infecting agent can be demonstrated in the blood during the early stages of the infection. When this is the case, the propagation is usually by means of the blood stream, the localization and multiplication depending on the most suitable environment.

In this case the affected cells were of diverse type, but in general they were predominantly cells of epithelial nature. The apparent precedence of the involvement of the endothelial cells in many of the affected areas suggests that the blood stream was the route of propagation. The predominance of the nuclear lesion is of interest in view of the possible identification of a common nuclear substance necessary for the multiplication of the virus and having a higher concentration in some cells than in others.

The extensive involvement of the esophageal mucosa and the unaffected condition of the gastric and intestinal mucosa are of interest, as is also the general involvement of the transitional epithelium of the excretory system.

The study of the central nervous system was complicated by hydrocephalus and thrombosis of the superior sagittal sinus. It is well to mention the papers of Byers and Hass⁴ and of Bailey and Hass⁵ on the latter syndrome, because the pathologic changes in the brain substance following thrombosis of the dural sinus of minor degree may have been construed as specific encephalitis by previous investigators. Thrombosis of the dural sinus is not an infrequent complication of the acute infectious diseases of childhood and need not result in death. The clinical sequelae may be severe, and it is well to consider this complication when a child has an episode of cerebral symptoms in the course of measles or chickenpox.

Only a few fragments of dorsal root ganglions were available for study. A more complete examination of these ganglions would have been interesting in view of the possible relationship of herpes zoster and varicella. The presence of degenerating cells in the portions of dorsal root ganglions obtained and the fact that the majority of the adjacent cells were well preserved may indicate some localization in this region. The central nervous system material studied in this case was fixed in solution of formaldehyde U.S.P., and it is possible that had tissue fixed in Zenker's fluid (acetic acid) been obtained other specific cytologic changes might have been observed.

The giant cell formation has been attributed to amitotic division of nuclei. Proof of this has been noted in the occasional flattening of two nuclei against each other as if they had just divided and also elongation of other nuclei with thinning of the central portion as if a nucleus were separating. These changes could have been due to stresses occurring as the result of swelling of epithelial cells and accumulation of intercellular fluid.

4. Byers, R. K., and Hass, G. M.: *Am. J. Dis. Child.* **45**:1161, 1933.

5. Bailey, O. T., and Hass, G. M.: *Brain* **60**:293, 1937.

This study indicates that specific pathologic confirmation might be obtained for many of the clinical manifestations of the acute infectious diseases if a careful histologic study of all tissues were carried out.

SUMMARY

A case of varicella in which a complete postmortem study was made is presented because of the demonstration of lesions that were apparently specific in tissues other than the skin. Areas of focal degeneration, microscopically observed, are described in the esophagus, pancreas, liver, renal pelvis, ureters, bladder and adrenal glands. These lesions showed cellular changes similar to those described in the skin.

Gross lesions were seen in the oral mucous membrane and in the corneas. No sections were obtained from these areas. The fluid in the anterior chamber of each eye was cloudy.

The characteristic cellular changes in varicella consist of ballooning of the affected cell, rarefaction and vacuolation of the cytoplasm, rarefaction of the nucleus with peripheral dispersion of the nucleolus and of the nuclear chromatin, and the presence of a well defined acidophilic inclusion body in the nucleus. These changes were observed in the epithelial cells of the skin, esophagus, renal pelvis, ureters, and bile ducts. They were also present in vascular endothelial cells, endothelial cells of lymphatics, histiocytes, acinar cells of the pancreas and medullary and cortical cells of the adrenal glands.

The capillaries associated with the individual lesion were often entirely destroyed, with resultant thrombosis and hemorrhage. A study of the various types of lesions suggests that the virus propagates by the blood stream, localizes in tissues predominantly of epithelial nature, multiplies in cells at the expense of these cells and has a special affinity for the nuclear substance of the epithelial cells of the skin.

The early lesion is not associated with inflammatory cellular infiltration, but this occurs subsequent to vascular infarction and resultant necrosis of tissue.

MOUSE LEPROSY

CECIL KRAKOWER, M.D.

AND

LUIS M. GONZÁLEZ, B.S.

SAN JUAN, PUERTO RICO

Spontaneous leprosy in a wild brown mouse was described in a former communication.¹ An emulsion of leproma from this animal was inoculated intraperitoneally into white mice. Lepromatous material from these was used for subsequent transfer to albino rats and mice, employing subcutaneous or intramuscular and intraperitoneal routes of inoculation. Continued passage from rat to rat and mouse to mouse has been maintained for the past two years through three or four series. Both rats and mice of the ordinary albino inbred laboratory type are susceptible. Not in a single animal has the disease failed to develop. The gross anatomic similarities and the histologic differences of the experimental disease in its later stages in these two animals will be discussed.

It may be well to mention at first that varied attempts to culture the organism of mouse leprosy have failed. In guinea pigs and rabbits subcutaneous inoculations of lepromatous emulsions from infected mice have generally given rise to localized abscesses within a month. These have ruptured and healed or in some instances have remained unchanged—for as long as sixteen months in 1 guinea pig, which was then killed for other purposes. Well preserved acid-fast bacilli were still numerous in the purulent contents as well as in the parietal monocytes. Extension of the disease in these refractory laboratory animals has not been observed. Nor have local lepromas comparable to those in rats and mice been formed.

The character of the local and distant lesions following intramuscular or subcutaneous inoculations will be described first. It has been found that in order to obtain large nonulcerated lepromas in rats and mice deep intramuscular inoculations in the thigh are preferable. With subcutaneous inoculations ulceration occurs much sooner, and the life of the animal may be considerably shortened as a result of secondary infection. The evolution of the local leproma, as Lowe² has indicated, is considerably hastened if the inoculum is a heavy one. With a very light inoculum only a local small lesion may be obtained at the end

From the School of Tropical Medicine.

1. Krakower, C., and González, L. M.: *Science* **86**:617, 1937.

2. Lowe, J.: *Indian J. M. Research* **22**:187, 1934.

of a year or more. With 0.1 cc. of a heavy lepromatous emulsion, containing numerous acid-fast bacilli, for mice and 0.5 cc. for rats, deep intramuscular inoculations in the thigh yield large lepromas within five or six months. Subsequently, over these large tumorous masses, one or more ulcers may appear. With diffuse invasion of the muscle the hindlimb may become apparently shorter as a result both of fixation in flexion and of general atrophy of the noninvolved portions of the limb.

The local leproma in the rat is larger than that in the mouse, commensurate more or less with the difference in bodily size. In both animals there is progressive infiltration of the muscle, radiating from the site of inoculation, until the thigh in the greater part of its length and circumference is massively involved, the infection being restricted only by bone and to a degree by fascia and skin. Subsequently, peripelvic and pelvic tissues are involved as well, resulting in deformity and displacement of the external genitalia and anal orifice to the opposite side. There is also extension, chiefly subcutaneous, below the knee joint. The large nerve trunks are surrounded but are not appreciably directly involved. Microscopically, however, the perineurium and often the endoneurium of small peripheral nerve trunks both here and in lepromatous areas elsewhere are infiltrated by lepra cells. The veins seen coursing over the leproma after the skin has been reflected are often markedly enlarged, including the femoral and even to a degree the ipsilateral iliac vein. While the thin walls of smaller veins microscopically are permeable to the infiltration of lepra cells, which may extend to the endothelium, arteries and arterioles are spared. In rare instances the circulation of blood to the distal portion of the hindleg has been sufficiently impaired to produce gangrene and autoamputation.

The leproma in the rat is generally quite firm and has a yellowish tinge. There may be smaller or larger liquefied areas, but these are not nearly as extensive as in the mouse. It characteristically possesses somewhat of an alveolated structure when cut in one plane and a more cylindric or tubular structure in the opposite plane. The latter largely conforms to the direction of the fibers of the muscles of the thigh. Delicate grayish depressed bands separate these areas of infiltration. The centers of the alveolated areas or of the cylinders are almost regularly softened and may appear depressed or excavated. They are yellower than the firmer peripheral portions.

In the mouse the leproma is more homogeneous in its solid portions, with fewer and less regular fibrous septums. Liquefaction is more marked, and a white milky material, involving at times the greater extent of the leproma, may flow out under pressure when the leproma is incised. This fluid contains rather few intact lepra cells, many polymorphonuclears with bacilli and myriads of acid-fast bacilli, which lend to a smear stained by the Ziehl-Neelsen method a deep red color.

The ipsilateral popliteal and iliac lymph nodes in both rat and mouse are the first to enlarge. In later stages they become very large and are occupied by white lepromatous tissue. The inguinal lymph nodes on the inoculated side are mildly or markedly involved, depending on the degree of infiltration of the superficial fascia. They are the ones to be

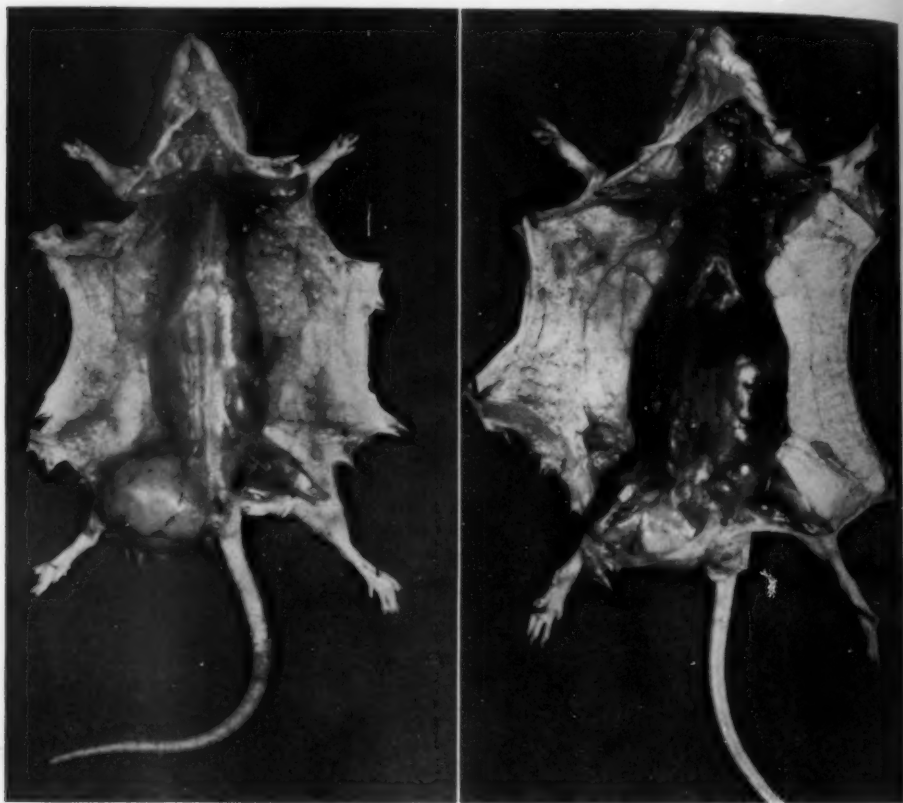


Fig. 1.—Left: Rat nine months after intramuscular inoculation in right thigh. Note the large lepromatous tumor of the thigh, the right inguinal lymph gland, slightly larger than the left, and the enlarged axillary and elbow lymph nodes. There are scattered discrete cutaneous nodules, best seen over the left side. The rat is shown about one-third the normal size. Right: Mouse nine months after inoculation of the right thigh. As in the rat, there is a large lepromatous tumor of the thigh. The right inguinal lymph node is considerably larger than the left. The axillary lymph glands are greatly enlarged. There are many small cutaneous nodules, which are largely obscured by highlights. A few can, however, be identified along the superficial lateral thoracic veins and the skin of the left cervical region. The mouse is shown about two-thirds of the natural size.

first and most markedly involved when the inoculations are made into the subcutaneous tissues of the thigh. In later stages of the disease practically all lymph nodes are affected, including the contralateral ones

described, as well as, bilaterally, elbow, axillary, submaxillary and cervical, also mediastinal and abdominal nodes. The peripheral lymph nodes particularly stand out sharply in view of the general marked emaciation of the animal in the very late stages. If not seemingly involved in gross, these nodes have shown varied degrees of microscopic lepromatous infiltration. The mode of infiltration from the periphery inward bespeaks lymphatic extension, and for those distant from the primary site of inoculation, extension either directly from lymph node to lymph node or from secondary hematogenous foci to adjoining lymph nodes. The latter may be exemplified by the hematogenous distribution to the lung with secondary involvement of the peribronchial or mediastinal lymph nodes. It is also of interest to note how frequently capsular and pericapsular tissues of involved lymph nodes are infiltrated by lepra cells.

In reflecting the skin widely both ventrally and dorsally, by careful inspection one may frequently see in both rat and mouse small fascial and cutaneous lepromatous nodules. They may be few or many and of greater or lesser degree of vascularity. They occur more frequently in cervical, interscapular, subaxillary and lateral costal areas. At times these small nodules present narrow indurated and infiltrated cords proceeding from them, which are presumably indicative of lymphatic spread. Microscopically, in these late stages sections of skin from varied areas reveal either slight or more massive subfascial or submuscular and cutaneous involvement. The spread appears to be mainly along larger subcutaneous lymphatics, with subsequent extension to the corium of the skin. Hematogenous localization cannot, however, be excluded. In a few of the infected mice these distant skin lesions were grossly ulcerated, resembling those in the spontaneous disease.

There is usually wide visceral involvement. The heart has been frequently involved, presenting white glistening epicardial nodules. Microscopically, myocardial and subendocardial lepromatous foci of greater or lesser extent have been found in both rat and mouse. The lungs too have been affected with considerable regularity, far more so in the rat than in the mouse. Whitish gray pleural nodules, at times with more opaque or greenish centers, could be readily identified in the rat. These sometimes assumed the appearances of miliary tubercles. Similar lesions could be seen in sectioned surfaces of the lungs but were frequently obscured in the rat by atelectasis and pneumonia secondary to bronchiectasis. Histologically, the degree of lepromatous infiltration is more marked in the rat than in the mouse, particularly in the atelectatic portions. In general the localization is peribronchial and perivascular, involving irregular pulmonary fields and the pleura in instances. The spleen generally reveals no definite lesions grossly, but

scattered small foci or discrete lepra cells in follicles and pulp are encountered microscopically. This is true in spite at times of enormous enlargement of the spleen associated with extensive ulceration and secondary infection of the lepromatous tumor. The liver by contrast is either scarcely at all or slightly involved in the rat, whereas in the mouse goodly involvement most always occurs. In the instances of milder hepatic infiltration groups of lepra cells are generally centered about central hepatic veins, occasionally involving the wall of the vein with polypoid-like projections into the lumen. The greatest degree of lepromatous infiltration was found in a mouse in which the liver was hugely enlarged, firm and pale, resembling in all respects an amyloid liver. The iodine test was negative. Microscopically, there was diffuse and extensive infiltration by lepra cells, outlining narrow cords of remaining liver tissue. The pancreas and kidney have been rarely affected. Some lepra cells have been found in the adrenals, particularly at the junction of cortex and medulla. The uterus has been almost constantly involved to a greater or lesser degree in both rat and mouse. The gastrointestinal tract has been spared except for perirectal infiltration due to extension from the lepromatous tumor of the thigh. The bladder, vagina or prostate and urethra have been involved in the same way as the rectum. There have been only few males in the series, and in these there was no definite testicular involvement. The tongue has shown microscopically almost constantly some subepithelial and deeper lepromatous foci. Rarely the mucosa of the larynx or trachea has been the seat of some infiltration, but foci in the adjoining fatty and muscular tissues have been common. The parotid and submaxillary salivary glands have been frequently involved, seemingly in part by direct extension from the infiltrated capsules and surrounding alveolar tissues of the adjoining lymph nodes. The thyroid and brain have always been spared. The thymus was involved in one instance, in which it had failed to undergo complete involution. The eyelids have frequently been affected, and the choroid of the eye occasionally. The nasal mucous membrane histologically has shown no lepra cells in the few instances in which it was examined. Bone marrow (including at times nasal and cranial bones) has been regularly and widely the site of lepromatous localization, consisting either of scattered few cells or more massive cellular replacement. In fact, in the late stages in the rat the femoral bone marrow has been solidly white and lepromatous.

Intraperitoneal inoculations of a heavy emulsion (0.1 cc. for mice and 1.0 cc. for rats) have in the main pursued a similar widespread lymphatic and hematogenous distribution in the late stages, with marked abdominal and thoracic involvement in addition. The first intraperitoneal inoculations from the original mouse with the spontaneous disease to white mice gave rise to large single or multiple abscesses with cheesy

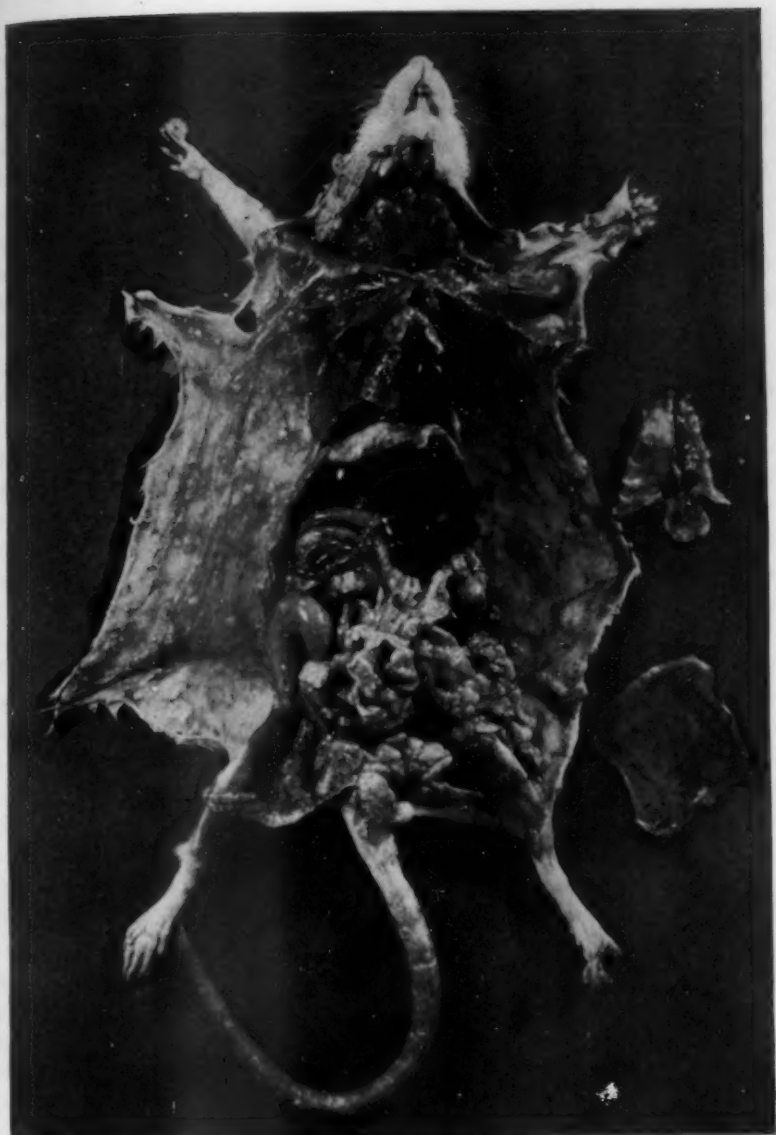


Fig. 2.—Rat inoculated intraperitoneally nine months ago. The omentum, mesentery, pelvic fatty pads and diaphragm are markedly thickened and replaced by lepromatous tissue. There are numerous large cutaneous nodules, pleural lepromatous plaques, and a discrete lepromatous nodule in the right auricle of the heart. To the right and above there is a retrosternal mass, and below the whitish flecked infiltrations of the anterior abdominal muscles are apparent. The rat is shown reduced two and one-half times.

and thick purulent contents, which caused varied degrees of local or more diffuse peritonitis. There was considerable matting of loops of intestine about these abscesses. The liver was often involved either by extension or hematogenously, resulting in one or more intrahepatic abscesses. Most of these mice died within two or three months. These large abscesses resembled in all respects both morphologically and in their rapid evolution those described in rat leprosy by Muir, Henderson and Landeman.³ The contents of the abscesses were made up largely of cellular detritus and polymorphonuclears. Few acid-fast bacilli were identified, but more of these were found within the monocytes and epithelioid cells of the capsule. Intraperitoneal inoculation of either the contents of the abscess or of the ground and emulsified wall produced similar lesions in mice, but subcutaneous inoculation of these into mice or intraperitoneal and subcutaneous inoculation into rats resulted in the usual characteristic lepromatous disease. Although no specific micro-organism could constantly be cultured from these abscesses, it is felt that they probably represented some type of secondary invader, probably carried over from the original wild mouse. When an emulsion from a nonulcerated leproma of the thigh of an otherwise healthy mouse was employed, no such abscesses were obtained but the characteristic lesions of intraperitoneal leprosy.

The peritoneal lesions first consist of tiny white flecks and granules, which seed mesentery, omentum and parietal peritoneal surfaces, including the diaphragm. These subsequently become confluent and in the rat omentum and mesentery may be converted into thick solid structures. Epididymal fatty lobules and mesometrium are markedly involved in both rat and mouse. These fatty membranes assume a solid, thick, whitish yellow appearance more often in the mouse and a more finely cobbled effect in the rat, with multiple small yellow necrotic areas. The abdominal muscles and diaphragm present a multiflecked appearance, and lepromatous tissue often infiltrates through their thickness, extending to involve in the former instance subcutis and cutis. Small subcutaneous lepromas are occasionally encountered at the site of inoculation.

In addition to these lesions in both rat and mouse there are smaller or larger pedunculated or sessile pearly white nodules attached to mesentery omentum or infiltrated fatty tabs which microscopically are the only indexes of any definite tendency toward progressive fibrosis noted in this disease. They are characterized in the late stages by dense hyaline practically avascular fibrosis enclosing greater or lesser numbers of free acid-fast bacilli as well as some atrophic lepra cells. Peripherally there are elongated lepra cells stuffed with bacilli. Progressive stages

3. Muir, E.; Henderson, J. M., and Landeman, E.: *Indian J. M. Research* **15**: 15, 1927.

in the development of the fibrosis can be traced from primary subserosal areas of lepra cell infiltration with progressive central autolysis or necrosis and the ingrowth or persistence of fibroblasts within these areas. Collagenous deposition occurs about these fibroblasts, leaving finally a densely fibrous but poorly cellular inner portion with only a narrow shell of viable lepra cells peripherally. Only in occasional marginal or subcapsular hepatic foci have such fibrotic changes been seen but elsewhere practically never, except vaguely in some of the subcutaneous nodules in the rat. No definite explanation can be offered for this, except possibly the enhanced vascularity of the surrounding serosal membrane or absorption of peritoneal fluid sufficient in either instance to maintain the viability and function of fibroblasts.

With regard to the abdominal organs there have been either miliary lepromatous granules or larger, flattish plaques over the capsular surfaces of the spleen, liver and kidney with some subcapsular extension in the two former organs but with otherwise little more deeper involvement than with the intramuscular route of inoculation. The uterus, urinary bladder and rectum, particularly the former two, have been markedly involved by direct extension from the peritoneal surfaces. In the case of the uterus the occasional more intense infection of the endometrium suggested the possibility of spread of infection from the lumen as well, the organisms being derived from heavily infiltrated ovarian or ovarian capsular surfaces. Whereas the serosa and to a degree the musculature of the stomach, duodenum and colon have been occasionally involved, the small intestine has been entirely spared, not even its serosal surfaces being affected.

In the thorax there has been usually massive retrosternal and at times paravertebral infiltration. The mediastinal lymph nodes have at times been hugely enlarged and tumorous. The heart and lungs have been no more involved than by the other route of inoculation. Except for in general greater infiltration and enlargement of abdominal and mediastinal lymph nodes and of the skin over the abdomen, the general widespread distribution of the disease as detailed in the description of the intramuscular route of inoculation has likewise occurred with intraperitoneal injections.

In general the histologic features of the disease repeat themselves with regularity in the different sites of involvement. In the rat the lepra cell is considerably polymorphous and more of epithelioid character. It has a fairly well defined cytoplasmic limiting membrane. The cytoplasm is generally more acidophilic than is the case of the lepra cell in the mouse. The nucleus is generally eccentric or at the periphery and is considerably polymorphous, more often kidney-shaped or centrally depressed and infolded, at times oval or round. Degenerative nuclear changes do not occur as frequently or as indiscriminately as in the



Fig. 3.—Upper: Rat. There is part of a necrotic area above. Note elsewhere the epithelioid character of the lepra cells and the numerous giant cells below. Lower: Mouse. The lepra cells are round or oval. The necroses are of cytolytic type, irregularly linear or more lacunar. Hematoxylin-eosin; $\times 78$.

mouse. The lepra cells are capable of stretching to a considerable degree, particularly where infiltrating between thick collagenous fibers, as in the skin or between smooth or striated muscular fibers. Subsequently they tend to round up as these structures atrophy and disappear. In consequence of their polymorphism, the lepra cells vary considerably in size, but the uninucleated cells generally measure from 10 to 25 microns in greatest diameter. Multinucleated giant cells, occasionally exceeding 80 microns in greatest diameter, are exceedingly frequent. These occur without any definite relationship to necrotic areas, as not infrequently in mildly infiltrated lymph nodes and quite irregularly and in unpredictable numbers, often being entirely absent. They are essentially of two types, but these are probably variants of the same mode of formation from uninucleated lepra cells. One form merges into the other. On the one hand, they are of characteristic Langhans type with a peripheral ring of nuclei and a central area of cytoplasm free of bacilli whereas the periphery harbors numerous bacilli. Diplosomes linearly arranged or massed are found in the clear central cytoplasm. On the other hand, there are giant cells having two or as many as twenty and more nuclei in the sectioned plane, arranged with less regularity in the cell and with either little or no cytoplasmic areas free of bacilli. One or at times two separate clusters of diplosomes are present. Mitoses in smaller uninucleated lepra cells are not at all infrequent, but have not been seen in multinucleated ones. In one instance, however, the nucleus of a uninucleated lepra cell was in a late anaphase stage of division, but this was unassociated with any evident division of the enlarged body of cytoplasm of the cell. It would appear in all that these giant cells are more the result of repeated mitotic divisions than of fusion or coalescence of single cells. If the latter were the case, it would be difficult to explain bacteria-free cytoplasmic areas such as occur in the Langhans type by the fusion of already bacteria-filled cells. The formation of such giant cells suggests rather a multiplication of nuclei and an increase of cytoplasm out of keeping with the proliferation of bacilli.

While the concentration of acid-fast bacilli in rat lepra cells is marked, there is some variation in different cells as to the degree of this concentration. For example, in some uninucleated and multinucleated cells they are very closely crowded, but somewhat less so in most other cells. In all instances, however, each bacillus occupies its own distinct place in the body of the cell and is separated from its neighbor by cytoplasm. There is slight but definitely greater spacing of the bacilli in the rat lepra cell than in the mouse. They appear to be longer, more distinct, less crowded and fewer per given unit of volume of cytoplasm in comparison with those of the mouse. It is for that reason that the rat lepra cell is more acidophilic in appearance. The lepra

bacillus in the rat is either straight or somewhat curved and is generally of more uniform length than that in the mouse. The mean length is more between 1.9 and 2.5 microns, whereas in the mouse there are greater variations and the mean lies more between 1.3 and 1.9 microns, i.e., the bacillus is in fact generally a little longer in the rat. In addition, in the rat there are well defined rosettes as first described by Cowdry and Ravold.⁴ These stellate aggregates of bacilli are generally found only in giant cells, largely those of Langhans' type. They have been seen in practically all sites of lepromatous involvement and have not necessarily been associated with necroses. They may, however, still be identified in necrotic areas where the cellular structure has completely disintegrated, and their own early disruption and loosening may be identified. The evolution of these stellate groups of bacilli can be traced from one or more small aggregates at the periphery particularly of Langhans' giant cells to large forms occupying practically the whole cell. They often have clear centers or are separated from the cytoplasm of the cell by a clear peripheral zone. In all instances there is no stainable cytoplasm between the individual bacilli, but only clear areas. They may readily be distinguished in different stains by either their negative or their positive characters. Thus in hematoxylin-eosin, Van Gieson's stain and phosphotungstic acid-hematoxylin they are unstained but stand out by contrast from the surrounding stained cytoplasm. With aniline blue they assume a distinct orange tinge. With scarlet red they are orange red, as every bacillus seems to take the fat stain more readily, while only the occasional bacillus elsewhere does so. In Masson's trichrome stain they are quite deeply stained by the alum-hematoxylin. By their grouping and compactness, they are readily identified in Gram, Giemsa and Ziehl-Neelsen stains. In general they are more readily stained by carbol fuchsin and less easily decolorized with acid alcohol.

These lepra cells in the rat fit themselves into a compact, solid, often nodular structure. There is a rich thick reticulum which surrounds practically every cell and which is considerably condensed and thickened in and about necrotic areas. To what extent this is a reactive process and to what extent it is the result of collapse due to the death of the lepra cells and subsequent shrinkage in those areas is difficult to determine. The vascular supply seems adequate for small lepromatous nodules or infiltrations but quite inadequate for the larger lepromatous masses. In consequence, while in occasional areas a single cell or groups of cells may show degenerative changes, in general there are well defined smaller and larger areas of frank necroses which are more of the nature of anemic infarcts. These are characterized by the persistence of the thickened reticular or collagenous framework, which assumes at times a

4. Cowdry, E. V., and Ravold, A.: *Puerto Rico J. Pub. Health & Trop. Med.* 14:95, 1938.



Fig. 4.—Upper: Rat. Dense reticulum surrounds every lepra cell. Lower: Mouse. Scanty reticulum separates columns or groups of lepra cells. Wilder's modification of Foot's reticulum stain; $\times 78$.

fibrinoid appearance without definite precipitation of fibrin. Within these necroses cellular debris and large numbers of liberated acid-fast bacilli are found. The outlines of lepra cells may, however, be retained, although the nuclei are rapidly lysed. Either the whole necrotic area is overrun by polymorphonuclears or more frequently, particularly in larger necroses, there is only peripheral polymorphonuclear infiltration. Within and adjoining these necrotic areas hyalinized vessels extensively infiltrated by fat are to be seen. There is little evident calcification or demonstrable iron. Liquefaction of the necrotic areas is infrequent. While there is no demonstrable fat in the lepra cell, it is frequently encountered in necrotic areas and within the infiltrating polymorphonuclears.

By contrast, in the mouse there are certain distinctive differences. The lepra cells except where infiltrating along fibrous or muscular planes are generally round or oval, much like a hypertrophied hematogenous monocyte. They are more uniform in size and on the average measure 15 microns in diameter. The nuclei are eccentric or at the periphery and are more often round or oval. The cells are sharply discrete with a well defined peripheral border. With ordinary hematoxylin and eosin stains they are more basophilic than the rat lepra cell. This as indicated heretofore is due to the intense concentration of bacilli, so that while they are still distinct and separate the amount of cytoplasm between them is very small indeed. The bacilli appear to be short and very closely crowded together within the cell, so that it is far more difficult to trace the length of any one of them than it is in the rat. Every well preserved lepra cell uniformly has this appearance of being stuffed with bacilli to capacity. It may be added too that the bacilli intracellularly assume a more orderly arrangement in the form of sheaves, bands or whorls than they do in the rat.

Uninucleated cells by far predominate. Binucleated cells are fairly frequent. Multinucleated cells exceeding 25 microns in diameter with more than five nuclei within the thickness of the sectioned cell are rare. Langhans' types of giant cells have not been encountered. In the giant cells there may be a very small area free of bacilli, within which grouped multiple diplosomes are to be identified. There are also frequently double diplosomes closely placed without a clear halo in binucleated cells and single diplosomes in uninucleated cells. Mitoses are very infrequent.

The lepra cells in the mouse are loosely grouped. There is a scanty reticular framework, often parallelly arranged, enclosing single cords of cells but not surrounding each cell. At times it is a little more abundant, particularly adjoining cytolysed areas. Vascularity is evidently poor. Necroses are frequent, but here there appears to be this distinction that even in small areas of infiltration, where the vascularity

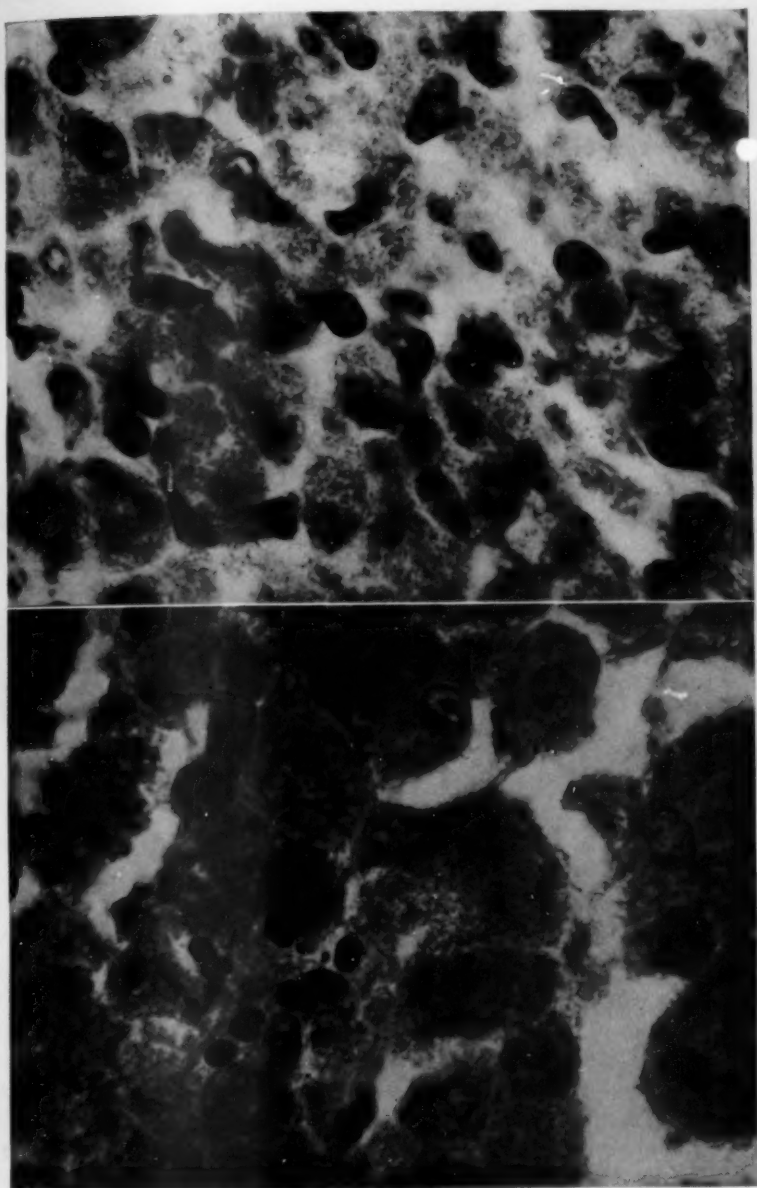


Fig. 5.—Upper: Rat. Lower: Mouse. To observe the difference in bacterial concentration within the lepra cells of rat and mouse note in the upper section, to the right, an intracellular group of bacilli surrounded by a clear halo resembling a globus. Giemsa stain; $\times 1,000$.

would appear to be adequate, as well as in those of more extensive character, the nuclei of the lepra cells often reveal the most bizarre degenerative changes. These vary from nuclear wrinkling and infolding to bizarre forms with multiple finger-like processes. Pyknosis is infrequent, but, even as in the rat, karyorrhexis and more often chromatolysis are the rule. These marked necrobiotic changes while undoubtedly

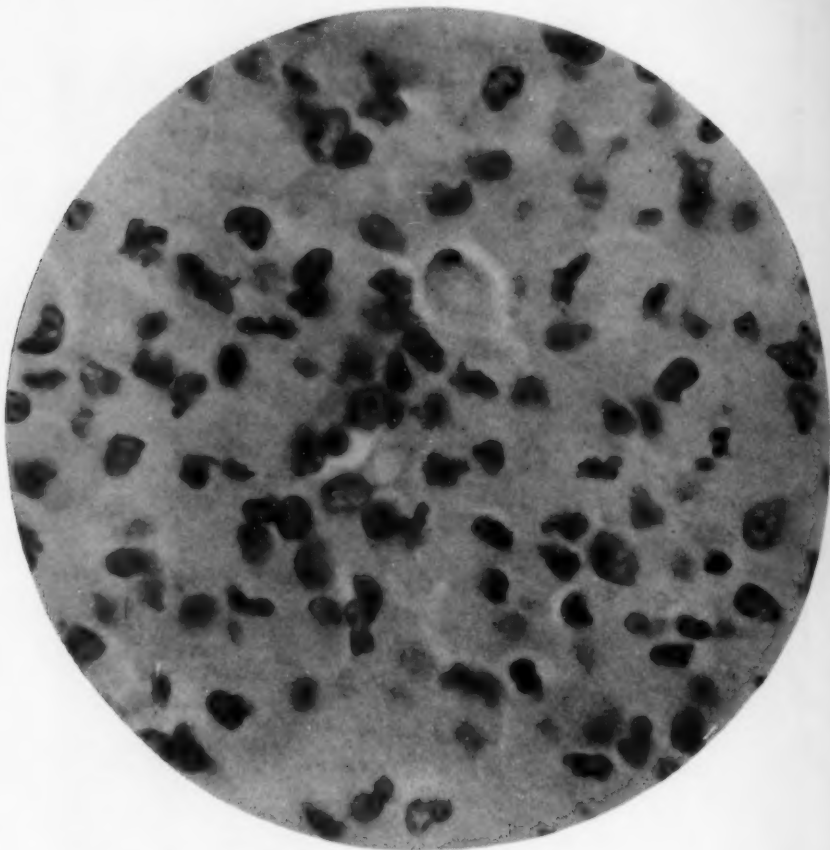


Fig. 6.—Mouse. This demonstrates the variety of nuclear deformities in lepra cells, the degree of which, however, cannot be appreciated in one plane of focus. Hematoxylin-eosin; $\times 1,000$.

largely due to poor vascularity may, however, also find their explanation in part in the unusual degree of bacterial parasitism of the cell. The necroses are quite different too from those of the rat. They are of frankly cytolytic type and of irregular distribution, assuming either a linear form or a more diffuse pattern, lending to the section a cribriform or lacunar appearance. In addition they may be circumscribed and

discrete or confluent and extensive. In these areas there is little remaining reticulum. The lepra cells are completely lysed, and there are merely masses of liberated bacilli, presumably in a nonstainable liquid medium with variable numbers of polymorphonuclears. There is little evident fat in these areas and none in the lepra cells. In common with lepromas in the rat, there are both diffuse and more focal perivascular aggregates of lymphocytes. Young histiocytes with moderate or slight bacterial content are also noted perivascularly.

Rosettes have not been encountered in the lepromas of mice. The closest approach to anything resembling either a rosette or a grouping of bacilli has been the occasional areas of clearing in viable lepra cells and more often in necrobiotic cells where the bacilli are somewhat loosely and more distantly spaced without any intervening or at times surrounding stainable cytoplasm. Occasionally both in sections and in smears nonnucleated oval structures with sharply limited borders packed with bacilli and without stainable cytoplasm have been seen in both rat and mouse. These appear to represent cells in which karyolysis or central cytolysis has occurred. The significance of these local intracellular or total cellular areas of clearing is quite obscure. In instances they remind one of small globi. The infiltrating polymorphonuclears harbor variable numbers of acid-fast bacilli, which in the rat in a few rare instances have been closely applied within the membrane of presumably a fatty vacuole. Otherwise they have been quite irregularly arranged or packed within the cell. In contrast with the more frequent chromatolysis of lepra cells, karyorrhexis is frequent among the polymorphonuclears.

There are a few additional observations that are worth recording. In both rat and mouse there has been little evident beading of the acid-fast bacilli as seen in sections, and no acid-fast granules have been identified. There is great variability in the ease of decolorization of the acid-fast bacilli, whether in the same cell or compared with the intracellular ones and those in necrotic areas. It is only by judicious care that practically all bacilli may be stained. In that way, bacilli that might have been regarded as having lost their acid fastness may adequately be shown to have retained it.

The phagocytic ability of the lepra cell is quite limited, and the only demonstrable evidences of such activity have now again been obtained in the presence of an ingested polymorphonuclear within a giant cell, of hemosiderin in lepra cells within lung and spleen and, in the original mouse with the spontaneous disease, of some melanin granules subepidermally.

It has also been of interest to note that the lepra cells which infiltrate the myocardium often possess nuclei with the characteristic chromatin arrangement of the so-called Anitschkow "myocyte," i.e., with a central

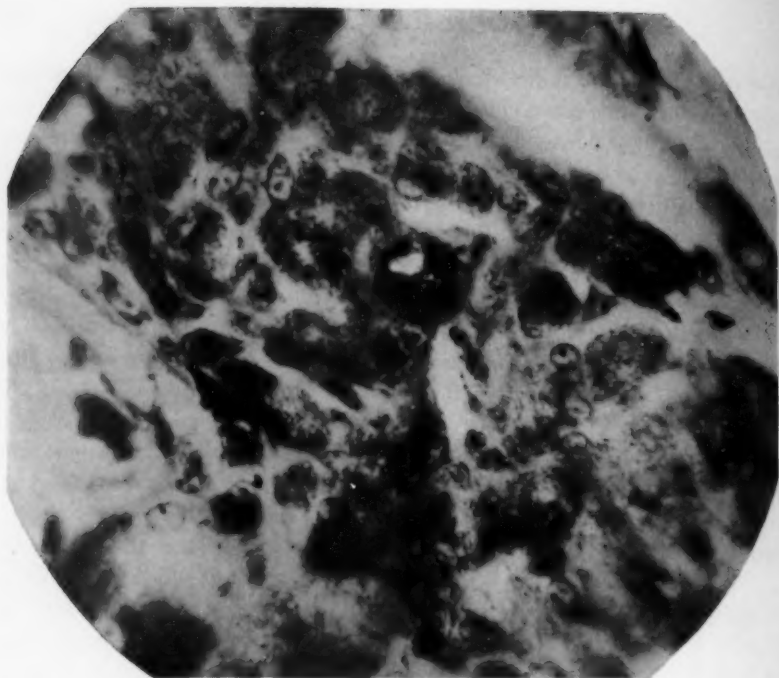


Fig. 7.—Upper: Rat. Note in the center, in a single large giant cell, three rosettes encroaching on a central bacteria-free area of cytoplasm. Lower: Mouse. Note the density of bacilli in the lepra cells as compared with those in the upper section and the arrangement in sheaves and whorls. Ziehl-Neelsen stain; $\times 1,000$.

thick bar of chromatin and radiating strands to the periphery. Inasmuch as it is generally agreed that the lepra cell is derived from the monocyte or the histiocyte, this would substantiate the view recently taken by Ehrlich and Lapan⁵ that cells with nuclei of this type occurring in both normal and pathologic hearts are likewise of the same origin. Not all the nuclei conform to this type, however. One gained the impression that they were more frequent in those cells at the periphery of the area of infiltration and in those lying between as yet intact myocardial fibers than in central areas of infiltration where the myocardial fibers had completely disappeared. Assuming that the more central lepra cells were derived by proliferation from peripheral ones after the destruction of the muscular fibers, this seemed to suggest that such nuclear configuration might be due to the mechanical factors of constant rapid contractions and dilatations of the heart acting on the cell rather than to any specificity on the part of the cell itself.

COMMENT

While there are reports in the literature on rat leprosy (Marchoux and Sorel,⁶ Sato⁷ and Row⁸ amongst others) to the effect that mice have been successfully inoculated with emulsions of rat lepromas, details as to the extent, degree or histologic features of the disease in the mouse are rather incomplete or, in the latter instance, lacking. Marchoux and Sorel and Sato stated, however, that the disease is less extensive or milder in the mouse. Recently Sellards and Pinkerton,⁹ in their attempts to transmit rat leprosy to mice, found the ordinary white mouse quite refractory to inoculation by subcutaneous routes although susceptible to intracerebral, intrasplenic and intraperitoneal inoculations. Pure strains of mice were, however, found to be more susceptible to subcutaneous injections. In the present instance the disease produced experimentally with mouse leprosy material, whether subcutaneously or intraperitoneally, has all the characteristics in ordinary inbred albino mice and rats of experimental rat leprosy in rats in which these same routes of inoculation are employed. The essential difference is one of size of the lesions, particularly of the lepromas at the sites of inoculation in the two animals, the size being smaller in the mouse than in the rat. This can be explained by the smaller dose of the inoculum employed for mice as well as by the limitation in growth determined by the differences in bodily size of the two animals. There is this additional difference,

5. Ehrlich, J. C., and Lapan, B.: *Arch. Path.* **28**:361, 1939.

6. Marchoux, E., and Sorel, F.: *Ann. Inst. Pasteur* **26**:675, 1912.

7. Sato, M.: *Jap. J. Dermat. & Urol.* **42**:149, 1937.

8. Row, R.: *Tr. Roy. Soc. Trop. Med. & Hyg.* **32**:497, 1939.

9. Sellards, A. W., and Pinkerton, H.: *Am. J. Path.* **14**:421, 1938.

that the spontaneous disease in the rat differs from the experimental one by the more frankly lymphoglandular or musculocutaneous involvement in the former, whereas the spontaneous disease in the mouse resembles to a degree the experimental one and cytologically has its exact counterpart in the experimentally infected mouse as well. In the mouse with spontaneous leprosy there was more marked involvement of the skin, such as that of the scalp and dorsal regions, with more diffuse involvement of forelegs and hindlegs than in the experimental disease. But the generalized involvement of lymph glands, skin and viscera in both are very much alike.

It is reasonable to suppose that the brown mouse with spontaneous leprosy may originally have become infected by contact with leprosy rats or their dejecta. Marchoux¹⁰ has described human infection with rat leprosy, and it seems possible that under natural conditions mice could also become infected. To what extent the bacillus of mouse leprosy differs biologically from Stefansky's bacillus of rat leprosy cannot be determined in view of the failure to culture both of these organisms. The greater infectivity of the bacillus of mouse leprosy for mice as compared with the strain from rats would suggest that there is some inherent difference.

While the experimental disease in rat and mouse with this mouse strain of leprosy is almost identical grossly in both, certain differences, particularly histologic ones, have been described (table). Some of these may be conveniently discussed.

The more frequent and greater degree of pulmonary involvement in the rat than in the mouse may perhaps be explained by the greater activity of the latter in its confinement to a small cage than that of the rat. This view that activity and in consequence more lively respiratory action with enhanced flow of blood and lymph may explain this difference is somewhat strengthened by the fact that more extensive lepromatous infiltration occurs in the poorly aerated and atelectatic portions of the lung so frequently secondary to suppurative bronchiectasis in older rats. By the same token, the striking absence of infiltration of the serosa or deeper layers of the small intestine in both rat and mouse following intraperitoneal inoculations, while it may be due to fewer available histiocytes in these tissues as compared with mesentery or omentum, may also perhaps be explained by the effect of active peristalsis and flow of lymph. It is admittedly difficult to correlate this view with the frequency and degree of lepromatous infiltration of the heart. While the differences in the liver may be due to the greater filtration of bacteria in the lung of the rat, fewer reaching the liver, this would scarcely be in keeping with

10. Marchoux, E.: *Ann. Inst. Pasteur* **37**:342, 1923.

the degree of involvement of the bone marrow. Here rather, the differences in size of the organ would have to be invoked; i. e., there would tend to be greater dilution in the distribution of bacteria in the larger-

Histologic Comparison of the Leproma of the Rat with That of the Mouse and with the Nodular Leproma of Man

	Rat	Mouse	Man
Cell type	Polymorphous and of epithelioid type with more often kidney-shaped and infolded nuclei	Round or oval and discrete, more like a hypertrophied hematogenous monocyte, with more basophilic cytoplasm and round or oval nuclei	Either of epithelioid or polymorphous, often elongated histiocytic type, frequently markedly vacuolated with poorly defined cellular boundaries; nuclei more often round or ovoid
Bacilli	Discrete and individual in the cytoplasm of the cell; grouping in the form of rosettes within giant cells; no globi	Discrete and individual in the cytoplasm of the cell; no "rosettes"; no globi	Either discrete or often grouped in packets and bundles or in compact clusters within intracellular and extracellular globi
Bacterial concentration per unit volume of cytoplasm of lepra cell	Marked but less than in the mouse	Exceeds that of the rat; bacilli more closely crowded, apparently shorter and more uniformly arranged in sheaves and bands than in the rat	Less than in the rat or mouse
Demonstrable lipoids	None except in necrotic areas and infiltrating polymorphonuclears	None in lepra cells and very little in necrotic areas	Quite abundant, particularly in vacuolated Virchow cells
Multinucleated giant cells	Frequent, with variations up to the typical Langhans type of giant cells, with twenty or more nuclei in one sectioned plane	Less frequent than in the rat, with rarely more than five or six nuclei in one sectioned plane; no giant cells of the Langhans type as seen in the rat	Quite frequent as seemingly fused vacuolated Virchow cells; Langhans' type of giant cell rarely encountered in nodular type of leproma
Mitoses of lepra cells	Fairly frequent	Rare	Present but variable as to frequency, generally infrequent
Necrobiosis of lepra cells as manifested chiefly by nuclear changes	Less frequent than in the mouse	Generally present to a marked degree	Generally slight
Necrosis	Frequent and well circumscribed, of anemic infarct type	Very frequent, of cytolytic and liquefactive type, involving at times a greater bulk of the leproma or assuming linear, lacunar or cribriform appearances	Generally absent
Reticulum	Less abundant than in man but distinctly more than in the mouse, surrounding practically every lepra cell	Comparatively scanty, more often limiting cords or groups of cells	Thick, abundant and closely meshed
Infiltrating cells of other types	Moderate numbers of lymphocytes and some plasma cells perivascularly or in focal nodules; many polymorphonuclears about and within necrotic areas	Much as in the rat	Lymphocytes and plasma cells are generally scattered throughout, with very few polymorphonuclears

sized liver of the rat than in that of the mouse, assuming that the Kupffer cells behave similarly in both.

Modifications in shape of lepra cell, amount of reticulum and character of necroses would appear to be inherent in the type of response

called forth in the two different types of animals. The first and last of these may well be dependent on the reticular response; i. e., the greater pleomorphism and compactness of the lepra cells in the rat may in great part be occasioned by the more abundant reticulum. It may also be that the rat lepra cell, less burdened by bacilli, may be more mobile than that of the mouse. Also necroses may more readily become liquefied where such reticulum is scanty, as in the mouse, and not restrained by one which becomes considerably condensed when necrosis occurs, as in the rat, assuming that autolysis and heterolysis are equally active in both.

The marked degree of bacterial concentration in the lepra cell of the mouse would indicate an unusual degree of adaptability of the micro-organism of mouse leprosy to the cell. Whether it occurs to a similar degree with the rat strain of leprosy in mice has not been reported. The bacterial concentration in the rat lepra cell with this strain of mouse leprosy is in keeping with that depicted in infections of the rat with the rat strain. In both rat and mouse infected monocytes or histiocytes (see Oliver,¹¹ Henderson,¹² Lowe¹³ and Pinkerton and Sellards¹⁴ for the origin of the lepra cell) hypertrophy in keeping with the increase of bacilli within them. It would appear that a greater degree of cellular hypertrophy occurs in the rat in response to the increase in numbers of bacilli than in the mouse; i. e., more cytoplasm is formed to separate and enclose the individual bacilli as they multiply, taking into account too the apparent greater length of the bacillus in the rat.

Inasmuch as these uninucleated cells have an ultimate limited capacity for growth there are in all less bacilli per given unit of area or volume of cytoplasm in the lepra cell of the rat and generally more in the whole of the lepra cell of the mouse as compared with a similar-sized cell in the rat. This greater capacity for hypertrophy as well as multiplication of infected cells in the rat is also evidenced by the greater frequency of mitoses and the abundance of giant cells, many of which, as in the Langhans type, have a large central body of cytoplasm free of bacteria. This would imply that with multiplication of nuclei a larger increase in cytoplasm can occur, out of proportion to the increase of bacilli, resulting in a stable cell with more free cytoplasm in which further general growth of the bacilli is restricted. The Langhans giant cell here can scarcely have the same significance as the similar type of cell occurring in tuberculoid leprous lesions in the human body, in which there are few or no demonstrable acid-fast bacilli. One gains the impression that this type of giant cell in the rat probably has little to do with destruction of the

11. Oliver, J.: *J. Exper. Med.* **43**:233, 1926.

12. Henderson, J. M.: *Indian J. M. Research* **16**:1, 1928.

13. Lowe, J.: *Internat. J. Leprosy* **5**:311 and 463, 1937.

14. Pinkerton, H., and Sellards, A. W.: *Am. J. Path.* **14**:435, 1938.

bacilli or reaction to their broken-down products. It is precisely so often in these cells that the rosettes take their origin and, as they grow, come to occupy more of the central bacterial free body of cytoplasm. These rosettes resemble bacterial microcolonies. Their absence in the mouse may be due to the fact that for their formation at least two requisites may be necessary, viz.: (1) a stable cell, i. e., one fixed in its ultimate size, with a convenient amount of free cytoplasm and with little further general growth of bacteria within it; (2) some modification of one or more bacilli at the periphery causing them to grow unrestrained. In the mouse the first of these requisites is scarcely met, for the multinucleated cells that occur are all stuffed with bacilli, and giant cells of the Langhans type or its close variant, as in the rat, must be very rare indeed, if they occur at all.

It may be added finally that the difference between the human lepra cell and that of the rat or mouse probably represents the cytologic expression of a disease with a varied flux, capable of complete resolution at times in the former and of relentless progression in the latter, at least in its experimental phases. The frequent marked vacuolated nature of the human lepra cell, its lipoid content, the grouping and clustering of bacilli into bundles and globi must represent chemical and physical forces as yet little understood but presumably capable of modifying the disease. Such changes are practically entirely absent in the lepra cells of rat or mouse.

SUMMARY

Ordinary albino mice and rats are susceptible to infection with a bacillus of mouse leprosy originally derived from a brown mouse with the spontaneous disease. Subcutaneous, intramuscular and intraperitoneal routes of inoculation were employed. In both animals the resulting experimental disease is entirely comparable to that produced in rats by Stefansky's bacillus of rat leprosy. The lepromas in the rat and mouse differ in size, in some of their gross features and particularly in their histologic and cytologic structure. In view of the apparent greater infectiousness of the bacilli of mouse leprosy for mice as compared with that of the rat strain it is felt that there is probably some inherent biologic difference between the two.¹⁵

15. Since this paper was submitted for publication G. L. Fite (Pathology of Experimental Rat Leprosy, in *Leprosy*, Bulletin 173, Federal Security Agency, Public Health Service, National Institute of Health, 1940) has described the cytologic structure of the lesions produced in ordinary laboratory mice with a strain of rat lepra bacilli. These observations agree essentially with ours on lesions in mice with mouse leprosy.

PATHOLOGY OF ACUTE AND OF HEALED EXPERIMENTAL PYELONEPHRITIS

G. K. MALLORY, M.D.

A. R. CRANE, M.D.

AND

J. E. EDWARDS, M.D.

BOSTON

Pyelonephritis has been recognized for years as an acute inflammatory disease involving the renal parenchyma and pelvis. It has become increasingly important to the internist through the works of Longcope and Winkenwerder,¹ Weiss and Parker² and others, who have demonstrated that it must be considered not only as a disease manifesting itself by signs of an inflammatory process but also as one which in its chronic and healed stages is an important cause of hypertension and renal impairment. Weiss and Parker² also pointed out that pyelonephritis, chronic or healed, is a common cause of Bright's disease, the cases forming a larger proportion of that group than cases of glomerulonephritis. They found that over a period of five years at the Boston City Hospital the diagnosis of some form of pyelonephritis was made 272 times, while that of some form of glomerulonephritis was made only 106 times. Staemmler's³ figures agree essentially; he reported that of 55 contracted kidneys, 27 were nephrosclerotic in type, 18 pyelonephritic and 10 glomerulonephritic.

It is therefore of considerable importance to be able to recognize accurately the end results of a pyelonephritic process in the kidney. In man it is difficult to trace step by step the development of such a process because of the difficulty of obtaining very early and healing lesions. Consequently we have thought it worth while to produce acute pyelonephritis in animals and to trace the renal lesions from the earliest recognizable process to the completely healed stage.

By so doing we have been able to support the criteria for the pathologic diagnosis of healed pyelonephritis set down by Putschar,⁴ Fahr⁵

From the Mallory Institute of Pathology, Boston City Hospital.

1. Longcope, W. T., and Winkenwerder, W. L.: *Bull. Johns Hopkins Hosp.* **53**:255, 1933.

2. Weiss, S., and Parker, F., Jr.: *Medicine* **18**:221, 1939.

3. Staemmler, M.: *München. med. Wchnschr.* **79**:2005, 1932.

4. Putschar, W., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1934, vol. 6, pt. 2, p. 441.

5. Fahr, T.: *Virchows Arch. f. path. Anat.* **301**:140, 1938.

and Weiss and Parker² and have obtained a correlation between the pathologic picture and the age of the lesions.

The majority of the studies carried out on experimental pyelonephritis which have been reported in the literature have been concerned more with the possible routes by which infection could be brought to the kidney than with the lesions produced. The histologic nature of the early lesions has been insufficiently described, and no attempt has been made to describe the healing or healed stages in any detail.

Three theories are ordinarily given to explain the mode of entrance of infection leading to human pyelonephritis. The first of these is that bacteria ascend from the bladder through the lumen of the ureter into the renal pelvis and renal parenchyma. The second explains the infection by an ascent of bacteria from the bladder by way of the lymphatics in and around the ureteral wall. The third maintains that the bacteria are carried to the kidney by the blood stream and pass from the blood stream into the renal parenchyma and pelvis.

These theories are still the subject of considerable controversy. Most of the experimental work has been carried out in an effort to prove or disprove them. Inasmuch as this is not our purpose we shall not discuss them in detail. David⁶ supported the first theory and reviewed the subject of ascending infections. Opposing the proponents of this theory are Brewer,⁷ Cabot and Crabtree,⁸ Wilson and Schloss,⁹ Chown,¹⁰ Lepper¹¹ and Draper and Braasch.¹² Stewart¹³ and Sweet and Stewart¹⁴ are advocates of the second theory.

Hematogenous infection is favored by Cabot and Crabtree,⁸ Wilson and Schloss,⁹ Chown¹⁰ and Brewer,⁷ on the basis of a study of human material. It is further supported by experimental work carried out by Sampson,¹⁵ Brewer,⁷ Hess,¹⁶ Lepper,¹¹ Kennedy,¹⁷ and Helmholtz and Beeler.¹⁸ Sampson,¹⁵ Brewer,⁷ Hess¹⁶ and Lepper¹¹ were able to produce acute pyelonephritis rather consistently by use of the hematogenous route. The method used by us is fundamentally similar to that employed by these four authors.

6. David, V. C.: *Surg., Gynec. & Obst.* **26**:159, 1918.

7. Brewer, G. E.: *J. A. M. A.* **57**:179, 1911.

8. Cabot, H., and Crabtree, E. G.: *Surg., Gynec. & Obst.* **23**:495, 1916.

9. Wilson, J. R., and Schloss, O. M.: *Am. J. Dis. Child.* **38**:227, 1929.

10. Chown, B.: *Arch. Dis. Childhood* **2**:97, 1927.

11. Lepper, E. H.: *J. Path. & Bact.* **24**:192, 1921.

12. Draper, J. W., and Braasch, W. F.: *J. A. M. A.* **60**:20, 1913.

13. Stewart, L. F.: *M. Bull. Univ. Pennsylvania* **23**:233, 1910-1911.

14. Sweet, J. E., and Stewart, L. F.: *Surg., Gynec. & Obst.* **18**:460, 1914.

15. Sampson, J. A.: *Bull. Johns Hopkins Hosp.* **14**:334, 1903.

16. Hess: *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **26**:135, 1913.

17. Kennedy, R. L. J.: *J. Urol.* **27**:371, 1932.

18. Helmholtz, H. F., and Beeler, C.: *Am. J. Dis. Child.* **14**:5, 1917.

In this paper we shall describe the gross and microscopic appearance of acute, healing and healed hematogenous pyelonephritis in the rabbit, the age of the lesions ranging from eighteen hours to eleven months.

MATERIAL AND METHODS

Rabbits weighing about 1.5 to 2 Kg. were used in the experiments.

The first step in the operative procedure was to ligate partially one ureter, usually the left, at about the midpoint in its course. Subsequently a saline emulsion of a growth of *Bacillus coli communis* was injected into the marginal vein of an ear. The injection was usually carried out either immediately following the ureteral ligation or twenty-four hours later. In a few animals the interval between the time of ligation and the time of injection was even greater, ranging from forty-eight hours to eight weeks.

Three strains of the organism were used. One was derived from the stool of a healthy rabbit, and two were obtained from the urine of patients with active pyelonephritis. The bacterial emulsion was prepared from a twenty-four hour culture on a plain agar slant. The dose averaged about 800,000,000 organisms.

The animals were killed with chloroform at intervals varying from thirty minutes to eleven months after the injection. Those animals in which we desired to study the healing process were reoperated on, on the fourth or fifth day. If an animal showed cortical renal abscesses, the ureteral tie was released and the animal allowed to live. Animals that showed no cortical abscesses were discarded.

Although no accurate count of the animals that were discarded was kept, definite pyelonephritis was obtained in about 75 per cent of the ones subjected to ureteral obstruction and injection of bacteria, and about 60 examples of representative stages of pyelonephritis were obtained. At times the virulence of the organisms became very low as a result of prolonged cultivation on agar, and at these times the percentage of pyelonephritis obtained was much lower.

When the virulence was increased by passage through animals, results similar to those first found were obtained. One strain became totally ineffective and had to be discarded.

GROSS CHANGES

Except for the changes resulting from acute hydronephrosis in the obstructed kidney nothing was visible grossly until about forty-eight hours after injection. At this time the pyramid showed minute round pale yellow areas, measuring about 0.5 mm. in diameter. These were more numerous in the obstructed kidney, but not infrequently one or two similar areas could be found in the pyramid of the unobstructed one. At this stage no gross change could be made out in the cortex or on the capsular surface of either kidney.

By the end of the fourth to fifth day definite and usually extensive pyelonephritis could be seen in the obstructed kidney. The organ was one and a half to two times its normal size. The pelvis and ureter above the level of obstruction were dilated and filled with thin yellow purulent material. On the capsular surface numerous abscesses, 0.5 to 1 mm. in diameter, could be seen. On cross section similar abscesses were found

scattered throughout the cortex and pyramid. Fine linear yellow to white streaking was also observed extending radially between the cortex and the tip of the pyramid. The parietal wall of the pelvis was thickened and edematous and not infrequently showed one or several abscesses. The distribution of the abscesses throughout the kidney as a whole varied somewhat. Sometimes their arrangement was distinctly focal, involving wedgelike areas (fig. 1 *B*); sometimes it was quite diffuse (fig. 1 *A*).

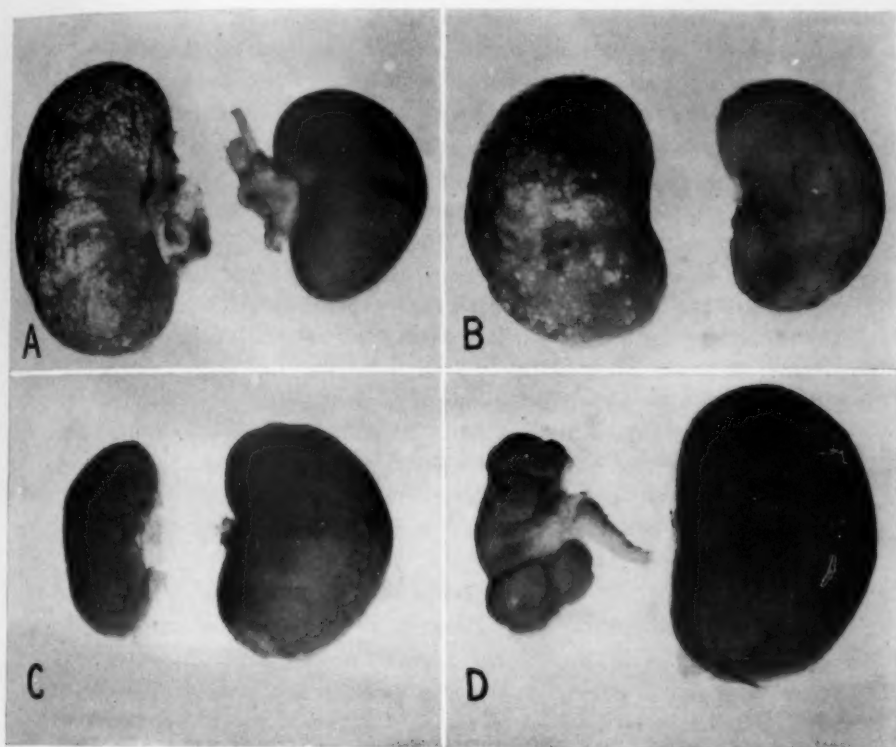


Fig. 1.—Photographs of both kidneys from each of 4 rabbits. Of each pair, the kidney on the left was obstructed for five days after intravenous injection of organisms. *A*, acute diffuse pyelonephritis five days after injection. *B*, acute, somewhat more focal pyelonephritis five days after injection. *C*, healed pyelonephritis with general contraction two and one-half months after injection. *D*, healed pyelonephritis with irregular coarse scarring eleven and one-half months after injection.

At no time was extensive pyelonephritis found in the unobstructed kidney. Sometimes one to several miliary abscesses were present in the tip of the pyramid, but never was there any involvement of the cortex or of the pelvis.

In the majority of the rabbits the obstruction was released at this stage. If this procedure was not carried out, the animals usually died at the end of seven or eight days, and a very extensive involvement of the obstructed kidney was found. The abscesses tended to become confluent and to extend outside of the kidney, forming a large perinephric abscess.

One animal in which the obstruction had been relieved at five days was killed eleven days later. The formerly obstructed kidney was still somewhat larger than normal, but the ureter and pelvis had returned to about the normal size and contained slightly cloudy fluid. Although at the time of reoperation definite cortical abscesses had been seen, at autopsy only a focal yellowish mottling of the cortex and pyramid was made out. The unobstructed kidney was essentially normal.

By the end of two months no definite further change seemed to take place. By this time the kidneys which had been obstructed and infected were markedly contracted. Usually they were about half their original size. They could more or less be divided into two groups from the appearance of their external contour. In the first and larger of these two groups the kidneys showed marked general contraction (fig. 1 C). The capsule peeled from what at first seemed to be a completely smooth surface; only when this surface was examined with a hand lens could it be seen to be very slightly granular. The cortex was uniformly reduced from the normal 3 to 4 mm. to about 1 mm., and the whole kidney was firmer and paler than normal. In color these kidneys were pale gray, in contrast to the reddish brown of those of the control side, and on cross section some fibrosis of the pyramid and fibrous thickening of the pelvis could be made out.

In the second group a similar contraction was present, but it was focal rather than diffuse (fig. 1 D). On stripping the capsule large irregular nodules of apparently normal renal substance were seen. These were surrounded by large, somewhat wedge-shaped areas of scarring and atrophy. On cross section the cortex was of about normal thickness in the noncontracted areas and very thin and atrophic in the scarred portions.

In none of these animals did the unobstructed kidney show any gross evidence of atrophy or scarring.

HISTOLOGIC CHANGES

The earliest histologic changes were found at about twelve to eighteen hours. These consisted only of masses of bacilli in the capillaries and venules of the pyramid, in vessels around the tubules of the cortex and in the capillaries of the glomerular tufts. These bacterial thrombi were present in both the obstructed and the unobstructed kidneys but were

much more numerous in the obstructed ones. They were larger and more conspicuous in the pyramids than in the cortex.

At twenty-four to forty-eight hours several changes were seen to be taking place. In the pyramid necrosis of the endothelium of the vessel around the bacteria occurred, and a reaction of polymorphonuclear leukocytes appeared about the vessel (fig. 2 *A*). In this way small interstitial abscesses were formed (fig. 2 *B*). In the same kidney other, apparently older lesions were often present in which the abscess had enlarged, caused necrosis of the epithelium of the adjacent collecting tubule and rupture of its contents into the lumen of the tubule. Interstitial abscesses were present also just beneath the pelvic mucosa covering the pyramid (fig. 2 *C*). These in similar fashion could be demonstrated to have caused necrosis of the pelvic mucosa and in this way to have ruptured into the lumen of the pelvis.

In the cortex at this stage an identical but much less marked process was found around a few venules. However, careful examination of the glomeruli and convoluted tubules in slightly older stages of the infection revealed a much more important process by which invasion of the cortex was initiated. Between forty-eight and sixty hours after the injection of organisms groups of bacilli were found in the capsular space of scattered glomeruli (fig. 3 *A*). In the same sections other glomeruli showed inflammatory processes. These varied from accumulations of polymorphonuclear leukocytes in the capsular space to necrosis and infiltration of the whole glomerulus, so that a small spherical abscess was formed (fig. 3 *B* and *C*). At times the inflammation could be demonstrated to have passed through the capsular basement membrane and in this way to have produced periglomerulitis. The origin of this inflammatory process seemed to be from the clumps of bacteria already described as present at twenty-four hours in the capillaries of the glomerular tuft.

At the same time a similar process was found in the convoluted tubules. Various stages could be seen in these also. Some tubules were essentially normal except for the presence of bacilli (fig. 4 *A*), others showed necrosis of the epithelium and invasion of the necrotic cells by bacteria (fig. 4 *B*), and still others presented the first two changes plus a reaction of polymorphonuclear leukocytes around the tubule.

A direct continuity between the inflammatory process in the glomeruli and that in the tubules was difficult to demonstrate, but in a few instances a glomerulus and a small segment of the tubule originating from it could be found to show a similar change. This stage in which organisms without reaction could be demonstrated in the capsular space and neighboring tubules was evidently of short duration, as it was never found after seventy-two hours.

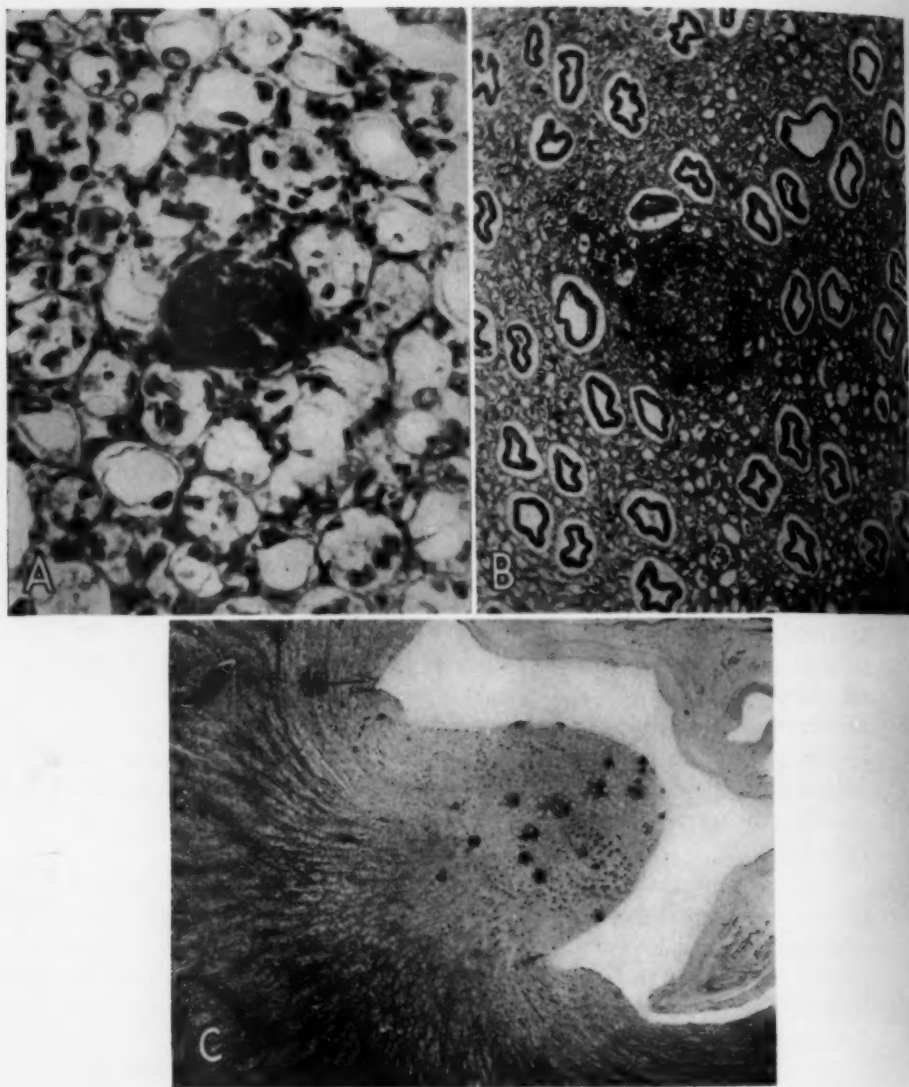


Fig. 2.—*A*, focus of organisms in a blood vessel of a pyramid, surrounded by an early reaction of polymorphonuclear leukocytes twenty-four hours after injection; $\times 800$. *B*, small interstitial abscess of a pyramid forty-eight hours after injection; $\times 400$. *C*, pyramid of an obstructed kidney forty-eight hours after injection; $\times 180$. Note many small interstitial abscesses, some of which lie just beneath the pelvic mucosa. Later these rupture directly into the renal pelvis. Zenker fixation; phloxine-methylene blue stain.

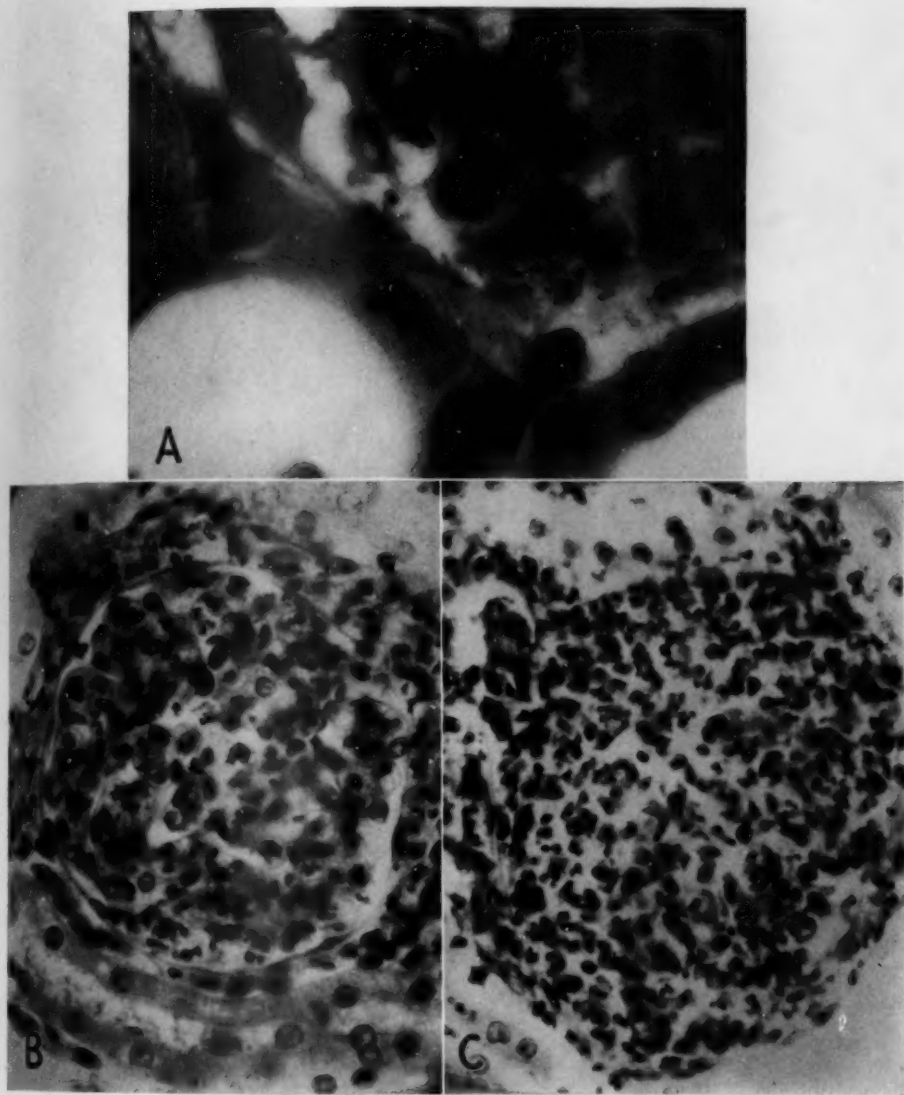


Fig. 3.—Early glomerular lesions sixty hours after injection. *A*, bacilli in capsular space; $\times 3,600$. *B*, acute glomerulitis and periglomerulitis; $\times 1,800$. *C*, abscess of glomerulus; $\times 1,800$. Zenker fixation; phloxine-methylene blue stain.

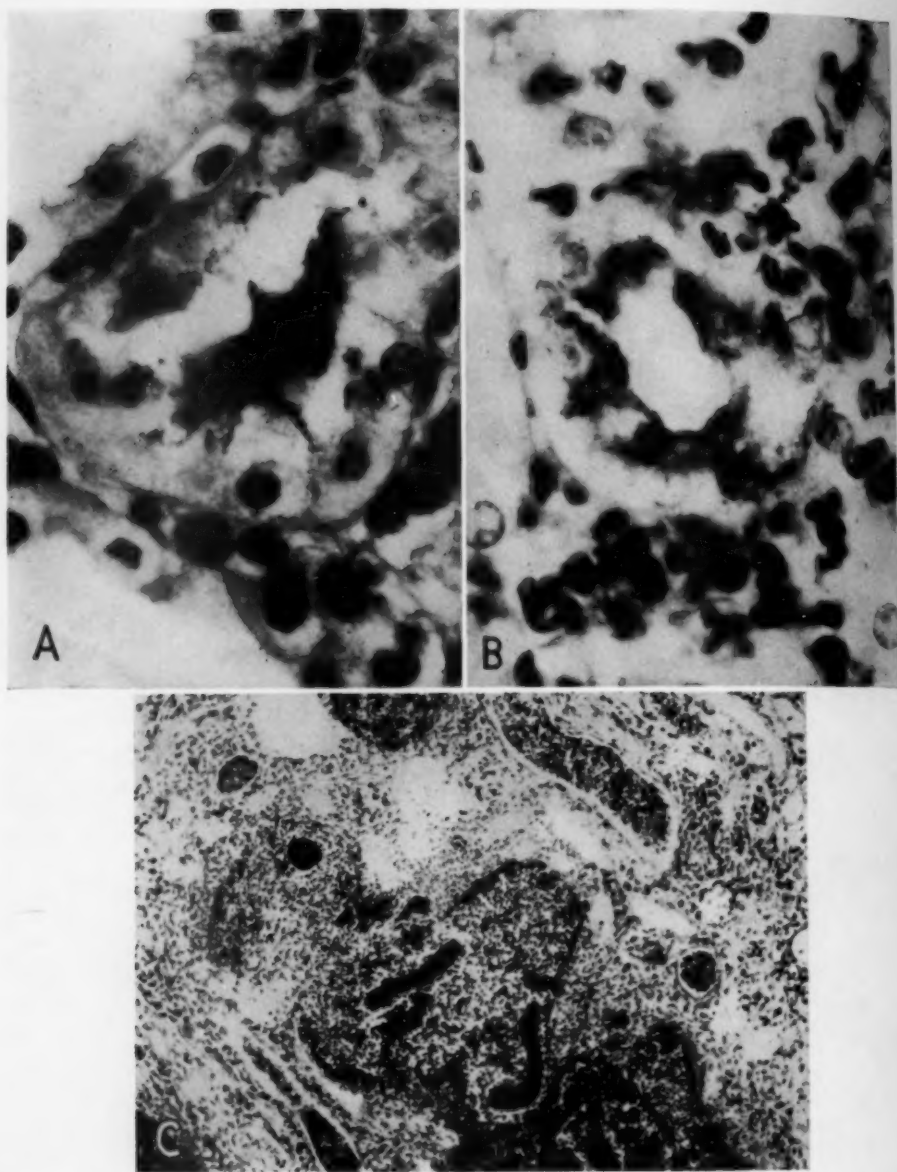


Fig. 4.—Development of cortical tubular lesions. *A*, bacilli in the lumen of a convoluted tubule sixty hours after injection; $\times 1,714$. *B*, necrosis of the epithelium of convoluted tubules, invasion of bacilli and early peritubular reaction of polymorphonuclear leukocytes sixty hours after injection; $\times 1,714$. *C*, large cortical peritubular abscesses four days after injection; $\times 200$. Zenker fixation; phloxine-methylene blue stain.

The problem of whether or not organisms can rupture into the glomerular space and pass into the tubules without causing diffuse inflammation of the glomerulus is of interest but difficult to solve. The fact that in later stages one can find apparently intact glomeruli surrounded by an inflammatory process suggests such a possibility.

We were never able to demonstrate a process similar to that just described in the unobstructed kidney. Although bacterial emboli were present here at twenty-four hours, organisms were never found in the glomerular capsular space or in the lumens of the convoluted tubules. One is forced to interpret these observations as meaning either that the bacilli do not rupture into the capsular space or that, if they do, they are washed out through the tubules too rapidly to be found.

By the end of the fourth or fifth day well established pyelonephritis was found involving the obstructed kidney. This was identical in every way with acute colon bacillus pyelonephritis in man. The collecting tubules remaining were somewhat dilated and filled with polymorphonuclear leukocytes. Similar cells were infiltrating the interstitial tissue, and in places small abscesses were present. In certain areas, although the tubules were filled with pus, the surrounding tissue was not infiltrated, suggesting that the infection in these areas had come down from the upper part of the nephron (fig. 5 *A*).

In the cortex there were large areas of abscess formation (fig. 4 *C*). These had apparently started in the glomeruli or convoluted tubules and spread outward. It was practically impossible to recognize in these abscesses any glomeruli as such, and this was interpreted as meaning that they had been completely destroyed. Scattered throughout the abscesses were necrotic masses of epithelium from the convoluted tubules. When the lumen of the tubule could be made out, numerous bacilli were still present in it, and these could be seen to have invaded the epithelium. The loops of Henle were similarly involved. Most of the collecting tubules were still intact and contained an acute exudate and organisms. These observations led us to the conclusion that during the period of acute inflammation many nephrons down to the point where they joined the convoluted tubules were completely destroyed.

At this period the unobstructed kidney presented a striking contrast. It showed little or no inflammatory activity. If small interstitial abscesses had formed in a pyramid, these were found to have ruptured into the adjacent tubules and to a large extent to have disappeared. At most a focus of a few polymorphonuclear leukocytes and organisms was found in the interstitial tissue and tubular lumens.

If at this stage the obstruction to the ureter was released, the subsequent histologic picture was that of a progressive and rather rapidly healing inflammatory process. By the end of two weeks after the injec-

tion of the organisms usually no bacilli could be demonstrated histologically or by culture. The acute inflammatory exudate was gradually replaced by a more chronic one, and a marked degree of fibrosis developed in the areas that had been involved in the inflammatory process. From here on, therefore, it seems advisable to trace the progressive changes as they affect the separate structures in the kidney rather than to attempt to describe each individual stage.

The descriptions as given apply to the originally obstructed kidney. In the unobstructed one the lesions quickly disappeared, and by the end of a month only minute foci of interstitial fibrosis remained as evidence that any inflammatory process had been present.

As has already been described, numerous glomeruli were completely destroyed and disappeared during the acute stage. Further glomerular change in the healing and healed lesions was not striking. Rarely a slight amount of pericapsular fibrosis was found. In only the oldest stage observed by us (eleven and a half months after infection) were any hyalinized glomeruli found. There was no focal scarring such as is found in healed focal embolic glomerulonephritis in man. Not infrequently, apparently normal glomeruli were found entirely surrounded by scarred atrophic tissue, suggesting the possibility that organisms might have passed through them to infect the associated tubules without causing any noticeable damage to the tufts themselves.

The progressive changes in the tubules were prominent and seem of importance in explaining the characteristic histologic picture seen in the healed lesions. As has already been described under the head of acute lesions, there was complete destruction of many of the convoluted tubules during the acute stages. Apparently this was of such a degree that regeneration could not take place. In the healed stages two months or more of age, convoluted tubules as such could not be recognized in any of the scarred areas.

Many of the collecting tubules in both the cortex and the pyramid were found to have survived. At five days they were usually dilated and filled with an exudate of polymorphonuclear leukocytes. Even after the release of the obstruction this exudate still remained, probably because the portion of the nephron leading to the tubules was destroyed and therefore no washing out of their lumens was possible. In the three and four week stages these polymorphonuclear leukocytes were still present, forming cellular casts in the lumens. In the six and eight week stages the leukocytes began to undergo a curious process of disintegration and fusion (fig. 5 B). At this time some of the collecting tubules were found to contain basophilic homogeneous colloid casts similar to those that are considered as a characteristic finding in human chronic or healed pyelonephritis. Other tubules contained fairly well preserved

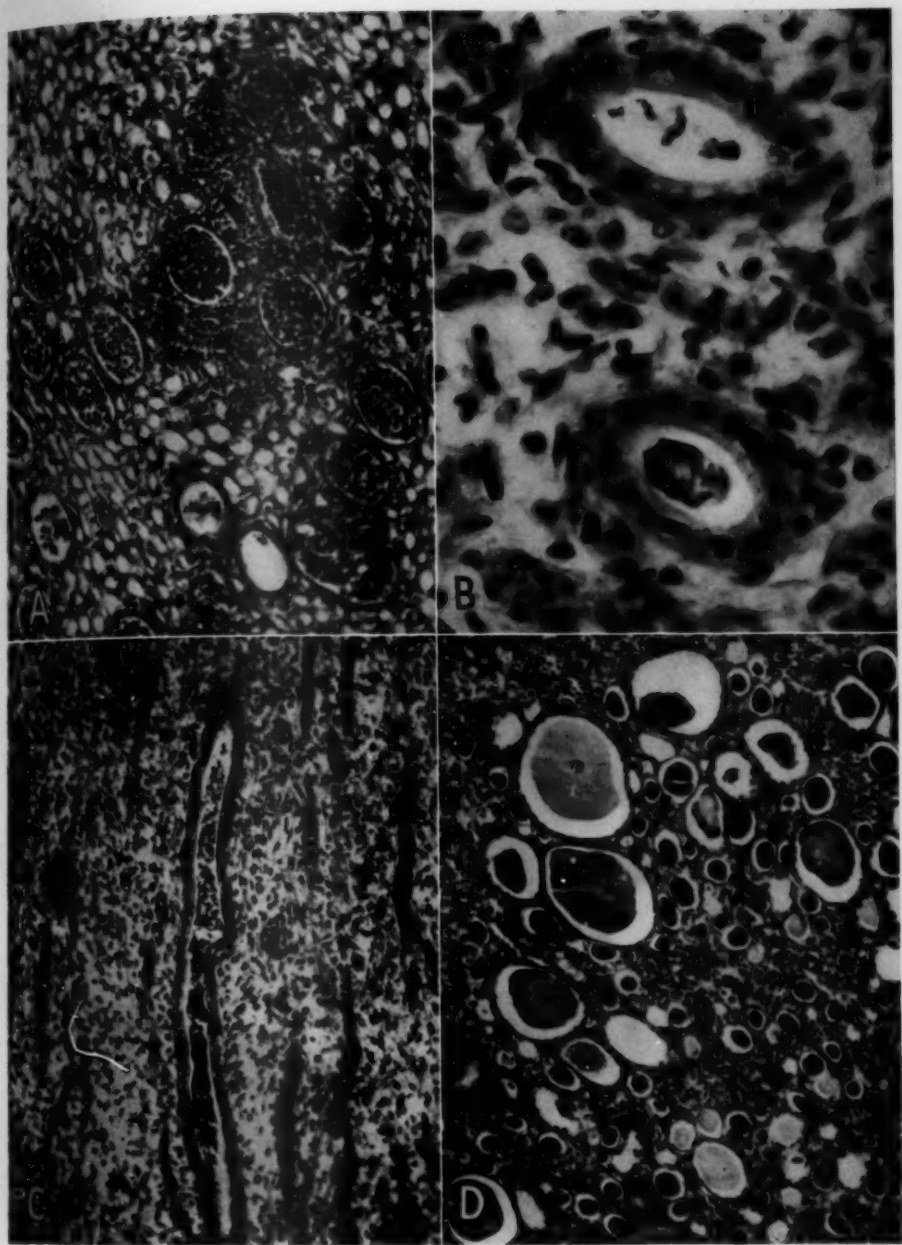


Fig. 5.—These photomicrographs portray the development of colloid casts. *A*, polymorphonuclear leukocytes in collecting tubules of a pyramid with interstitial reaction around some of the tubules five days after injection; $\times 400$. *B*, cross section of two tubules six weeks after injection; the upper tubule contains necrotic polymorphonuclears; in the other the leukocytes have fused to form an irregular network of blue-staining colloid material; $\times 800$. *C*, longitudinal section of a cast six weeks after injection; the lower portion has become homogeneous and colloid has formed; the upper portion shows necrotic appearing polymorphonuclears which are beginning to fuse; $\times 600$. *D*, tubules with completely formed colloid casts eleven and one-half months after injection; $\times 400$. Zenker fixation; phloxine-methylene blue stain.



Fig. 6.—Cross section through a whole kidney. Peripelvic fibrosis, focal lymphocytic infiltration around the pelvis and in the cortex, fibrosis of the pyramid and the cortex and dilated tubules containing colloid casts can be seen at three months; $\times 8$. Zenker fixation; phloxine-methylene blue stain.

polymorphonuclear leukocytes. In still others intermediate stages were present. In some tubules in which a longitudinal section of a cast could be found, different portions of the same cast presented a varied structure (fig. 5C). One portion would be made up of somewhat necrotic-appearing polymorphonuclear leukocytes. Other parts would consist of homogeneous colloid material and still others of a network of this colloid. The latter picture seemed to result from fusion of the nuclear material of the leukocytes. It seemed, therefore, permissible to interpret the blue colloid casts of healed pyelonephritis as a result of the disintegration of groups of polymorphonuclear leukocytes caught in the tubular lumen because of the destruction of the upper portion of the nephron.

During the acute stages numerous abscesses surrounded by an infiltration of polymorphonuclear leukocytes were present in the interstitial tissue. By the end of two weeks all but the largest abscesses had disappeared, and the infiltration was rapidly becoming one of lymphocytes and plasma cells rather than polymorphonuclear leukocytes. By the end of a month lymphocytes were about the only remaining inflammatory cells. These tended to occur in foci in the cortex and pyramid. They were often quite prominent adjacent to the pelvis. During this time a marked increase in the interstitial fibrous tissue also had taken place. This again was most prominent in the cortex in areas where there had been widespread destruction of glomeruli and tubules and in the pyramid, particularly toward its tip.

The peripelvic fibrous tissue showed a similar change. It became markedly thickened, large numbers of collagen fibers were laid down, and foci of lymphocytic and plasma cell infiltration appeared.

In the eleven and one half month period over which we have so far been able to follow our lesions the blood vessel changes have not been very prominent. During the acute stages thrombosis of some of the veins could be demonstrated. Although difficult to find, thrombosis of some of the smaller arteries and arterioles in the inflammatory areas could also be demonstrated. In the healed stages (two months to eleven and one half months) the arterioles in the scarred areas were definitely more prominent than those in the normal ones. This was the result of thickening of their media. In one healed pyelonephritic lesion four months old and another eleven and one half months old early hyperplastic arteriolar sclerosis was present.

COMMENT

Our experimental lesions are similar in most respects to pyelonephritis in man. Human kidneys showing healed pyelonephritis when considered in their gross aspect alone are of two types. The first type is

very coarsely scarred, the scars being irregular and wedge shaped; the second type is very small, evenly contracted and finely granular. The latter type is frequently associated with the condition known as renal dwarfism in children, and the condition is often spoken of as renal rickets. Experimentally we have produced a lesion identical with that of the first type of kidney and one very similar to that of the second save that no marked granularity of the surface is present. We feel that the difference in these two types of lesions can be explained on the basis of whether the involvement of the kidney was diffuse or focal during the acute pyelonephritis.

We have also reviewed histologic sections of human kidneys involved in acute pyelonephritis in an attempt to formulate some idea of which of the early lesions found by us in the experimental series could also be seen in the human disease.

The greatest difficulty in such a comparison is that in the majority of the human kidneys acute pyelonephritis is, by the time such kidneys reach the pathologist, entirely too far advanced for any satisfactory conclusion to be drawn as to its origin. Acute pyelonephritis in man *per se* does not ordinarily cause death or lead to surgical intervention within seventy-two hours, and early stages of the process are found only when pyelonephritis is incidental to or complicative of some other cause of death.

In the majority of the human kidneys which we examined acute pyelonephritis showed a picture which could not be distinguished from our experimental lesions of five or six days' duration. The cortical lesions consisted of fairly large abscesses in which foci of necrotic convoluted tubular epithelium were present. In such lumens as were left there were masses of organisms, mostly bacilli. Sometimes glomeruli undergoing acute inflammatory changes were found either in or adjacent to these abscesses. The pyramids showed marked acute interstitial infiltration, and the collecting tubules were filled with pus and organisms.

Our observations of organisms in the capsular space and convoluted tubules in the experimental kidneys agree with those described in early stages of human pyelonephritis by Cabot and Crabtree,⁸ in which bacteria were present in the capillaries of the glomerulus, in the capsular space and in the lumens of the convoluted tubules and in which also an acute inflammatory process could be found spreading out from the tubule to form abscesses. Wilson and Schloss,⁹ Chown¹⁰ and Kennedy,¹¹ studying the histologic appearance of early lesions, found early involvement of the interstitial tissue but did not describe any participation of the glomeruli in the process. A combination of the findings of Cabot and Crabtree and the latter group of authors results in a picture similar to our findings in the experimental lesions.

The lesions in one of our human kidneys duplicated our experimental lesions almost completely. This kidney was from a man of 65 years who died primarily as the result of a carcinoma of the rectosigmoid. The tumor had invaded the urinary bladder, forming a rectovesical fistula. The posterior wall of the bladder was involved by a fungating mass of tumor. No definite hydronephrosis was described, but possibly there was at least an acute obstruction of the ureters as they entered the bladder. The lumen of the bladder was filled with foul-smelling necrotic material.

In the kidney numerous glomeruli showed bacillary emboli in the capillaries of the tufts. Similar emboli were present in the larger venules and capillaries of the pyramids. In one glomerulus the capsular space was filled with organisms. Numerous convoluted tubules showed bacilli in their lumens, alone or with an associated acute peritubular reaction of polymorphonuclear leukocytes.

In 2 other kidneys, from patients with nephrolithiasis and chronic pyelonephritis, bacillary emboli were present in the glomeruli and pyramids. Around some of the latter a reaction of polymorphonuclear leukocytes was present, forming fresh interstitial abscesses.

The healed lesions found by us seem of some importance in that they emphasize the fact that the criteria used by Putschar,⁴ Fahr⁵ and Weiss and Parker² for the diagnosis of human healed pyelonephritis are entirely valid. The prominent features that we found were groups of tubules the lumens of which were filled with colloid casts staining from blue to pink, cortical fibrosis with focal lymphocytic infiltration and disappearance of glomeruli and tubules, and similar fibrosis and infiltration in the pyramids and around the pelvis. If our interpretation of the formation of these colloid casts is correct as given, their presence should be the most reliable criterion for the diagnosis of the healed lesion. According to this interpretation, they arise only when, at an earlier stage, not only the tubules have been filled with polymorphonuclear leukocytes but also destruction of the upper or lower portion of the nephron has occurred, imprisoning these leukocytes in the tubules. Acute pyelonephritis is probably the only lesion of the kidney which could produce such a combination of circumstances.

The importance of urinary obstruction for the development of our experimental hematogenous pyelonephritis is also worthy of comment. This has been emphasized by several workers, the foremost of whom have been Sampson¹⁵ and Lepper.¹¹ Lepper, using the method described earlier by Sampson,¹⁵ by Brewer⁷ and by Hess,¹⁶ confirmed the work of these three authors and demonstrated that urinary obstruction was a very important factor in the production of blood-borne pyelonephritis. After the ligation of one ureter, intravenous injection of colon bacilli

produced after four to six days definite acute pyelonephritis in the obstructed kidney and few or no lesions in the unobstructed one. These results agree exactly with ours.

It is interesting to note that Lepper also found that even transitory obstruction of the ureter was of importance in localizing the infectious lesions. In some of her experiments the ureteral obstruction was maintained for only thirty to forty-five minutes following the intravenous inoculation. In spite of the short duration of this obstruction the kidney so treated had a much more marked tendency to become infected.

Lepper referred to the work of Lucas¹⁹ in attempting to explain this increased susceptibility of the obstructed kidney to hematogenous infection. The latter author demonstrated a slowing of the venous circulation as a result of the rise of intraureteral pressure in hydronephrosis. It is very possible that this slower blood flow allows the circulating bacteria a chance to gain a more substantial foothold, leading to necrosis of the walls of the vessels and infection of the surrounding tissue.

In 1911 Brewer,⁷ considering the importance of urinary obstruction in the localization of hematogenous pyelonephritis, was also led to explain this phenomenon on the basis of vascular stasis. Consequently he investigated this problem in dogs. He found that in the presence of bacteremia obstruction of the renal vein or artery favored the development of acute pyelonephritis.

Kennedy,¹⁷ in 1932, reinvestigated the problem of the relationship of obstruction to the development of hematogenous colon bacillus pyelonephritis and concluded that obstruction had no effect on the localization of the infection. In his paper the technic used in experiments II and III was similar to ours. The unobstructed kidneys showed no abscesses, while the obstructed ones contained some inflammatory lesions. He explained these inflammatory lesions as not infectious but as secondary to hydronephrosis and considered them similar to the mild inflammatory changes described by Helmholz and Field²⁰ in experimental hydronephrosis.

In only one of Kennedy's animals did the obstructed kidney show changes which he felt were attributable to the injected organisms. In this kidney there were cortical abscesses, and the associated tubules were filled with pus. It is of importance to note that this animal lived only seventy-two hours after injection, and yet this was the longest that any of his series was allowed to survive. Brewer⁷ and Lepper,¹¹ who had used essentially the same technic, came to quite the opposite conclusion

19. Lucas, D. R.: *Am. J. Physiol.* **22**:245, 1908.

20. Helmholz, H. F., and Field, R. S.: *J. Urol.* **15**:409, 1926.

and found the infection predominantly in the obstructed kidney. Brewer, however, examined his animal after seven days and Lepper her animals after four to five days. In our experiments we had to wait four to five days to obtain any striking gross lesions. It seems probable, therefore, that if Kennedy's animals had survived a longer period of time he would have obtained different results and arrived at different conclusions.

SUMMARY

Unilateral pyelonephritis was produced by injecting colon bacilli intravenously into rabbits which had partial ligation of one ureter. Acute pyelonephritis similar to that seen in man was produced in the obstructed kidney in about 75 per cent of the animals. Extensive pyelonephritis was never found in the unobstructed kidney. By releasing the ureteral obstruction at the end of four to five days after the injection of bacilli healing of the pyelonephritic process was induced. In this way stages of acute, healing and healed pyelonephritis were produced.

The acute lesions were found to arise (a) from interstitial abscesses starting around clumps of bacteria in the small blood vessels and (b) as a result of organisms passing through the glomerulus into the tubules.

In the healed lesions thyroid-like areas of colloid casts were found. In earlier lesions a fusion of disintegrating polymorphonuclear leukocytes was found to lead to the formation of these casts.

The gross and the histologic appearance of the healed lesions confirm the criteria used by other authors and ourselves in the diagnosis of healed pyelonephritis in man.

CARCINOMA IN SITU OF THE STOMACH AND ITS BEARING ON THE HISTOGENESIS OF MALIGNANT ULCERS

TRACY B. MALLORY, M.D.

BOSTON

The observation of Hampton,¹ of the roentgenologic department of this hospital, that the major proportion of ulcerative lesions in the prepyloric inch of the stomach prove sooner or later to be cancerous has led to earlier and more radical surgical treatment of such lesions in the Massachusetts General Hospital for the past seven years. It is reasonable to expect that under such conditions certain cancers should have been resected at a much earlier stage of their development than would be the case where operation was delayed until all the classic roentgenologic evidences of malignancy were evident. Experience has proved this to be true. The pathologist has in fact been presented with several specimens in which the histologic diagnosis was most difficult since many of the cytologic features of malignancy were present but invasion was difficult to establish or could even be excluded. With increasing experience the surmise that certain of these lesions represented the earliest, noninvasive stage of cancer of the stomach grew into conviction. The evidence for this conclusion and the criteria for a diagnosis of carcinoma of the gastric mucosa in situ will be presented in part I.

Simultaneously with this study, an extensive survey of the genesis of peptic ulcer was in progress in the laboratory. The active phase of "peptic" ulceration was found to present a constant, characteristic, possibly pathognomonic picture, and the observation of identical types of ulceration in carcinomatous tissue was repeatedly made. Concurrently, in the roentgenologic department, under the effect of a standard medical regimen for ulcer therapy partial healing was noted in several ulcer craters which eventually were proved by resection to be cancerous. These observations appeared to offer experimental verification of the peptic character of the ulceration. The evidence to support these conclusions is presented in part II.

Throughout the seven year period over which this material has been collected numerous examples of so-called malignant ulcers or ulcer-

From the Department of Pathology and Bacteriology, Massachusetts General Hospital.

1. Hampton, A. O.: *Am. J. Roentgenol.* **30**:473, 1933.

cancers have accumulated, and we have been compelled to focus our attention on the highly controversial question of the relationship of ulceration and neoplasia of the stomach. The observations recorded in this paper offer new evidence bearing on this controversy, and their significance will be discussed.

I

From the many types of precancerous lesions which have been described it has gradually become apparent that a distinction can be drawn between those that *may* become malignant (such as arsenical keratoses and roentgen ray dermatitis) and others in which the evolution into invasive cancer appears to be constant, notably Paget's disease and Bowen's disease. The irreversible character of this evolution and the constant presence of certain histologic features common to malignant neoplasms but foreign to inflammatory reactions gradually aroused the suspicion that such conditions represented not merely potential foci of carcinogenesis but actual carcinoma in a preinvasive stage. This theory was crystallized by Schiller,² who, with repeated biopsies, followed similar lesions of the cervix over periods of several years till invasive carcinoma developed, an observation recently repeated by Younge.³ Broders⁴ independently developed the same concept, describing noninvasive stages of cancers of glandular tissues as well as of squamous cell origin, and coined the phrase "carcinoma in situ" to describe them. Such a diagnosis connotes an expression of faith on the part of the pathologist that a given segment of epithelium has undergone the initial stage of an irreversible sequence which can end only in frank invasive cancer. The time at which invasion may begin, however, is quite impossible to foretell and may be many years ahead (Schiller,⁵ seven years; Bowen's⁶ first case, nineteen years.)

Preinvasive carcinoma of the stomach occurs in two forms: the generally recognized polyp with malignant cytologic alterations, which has been well described by Borrmann,⁷ and a nonpolypoid form, very prone to ulceration, which occurs with significant frequency in the pyloric region. Lesions of this type have unquestionably been observed by others (Moszkowicz;⁸ Stromeyer⁹), but since the concept of car-

2. Schiller, W.: *Virchows Arch. f. path. Anat.* **263**:279, 1927.

3. Younge, P. A.: *Arch. Path.* **27**:804, 1939.

4. Broders, A. C.: *J. A. M. A.* **99**:1670, 1932.

5. Schiller, W.: *Surg., Gynec. & Obst.* **56**:210, 1933.

6. Bowen, J. T.: *J. Cutan. Dis.* **30**:241, 1912.

7. Borrmann, R., in Henke, F., and Lubarsch, O.: *Handbuch der speziellen pathologischen Anatomie und Histologie*, Berlin, Julius Springer, 1926, vol. 4, pt. 1, p. 812.

8. Moszkowicz, L.: *Virchows Arch. f. path. Anat.* **253**:511, 1924.

9. Stromeyer, F.: *Beitr. z. path. Anat. u. z. allg. Path.* **54**:1, 1913.

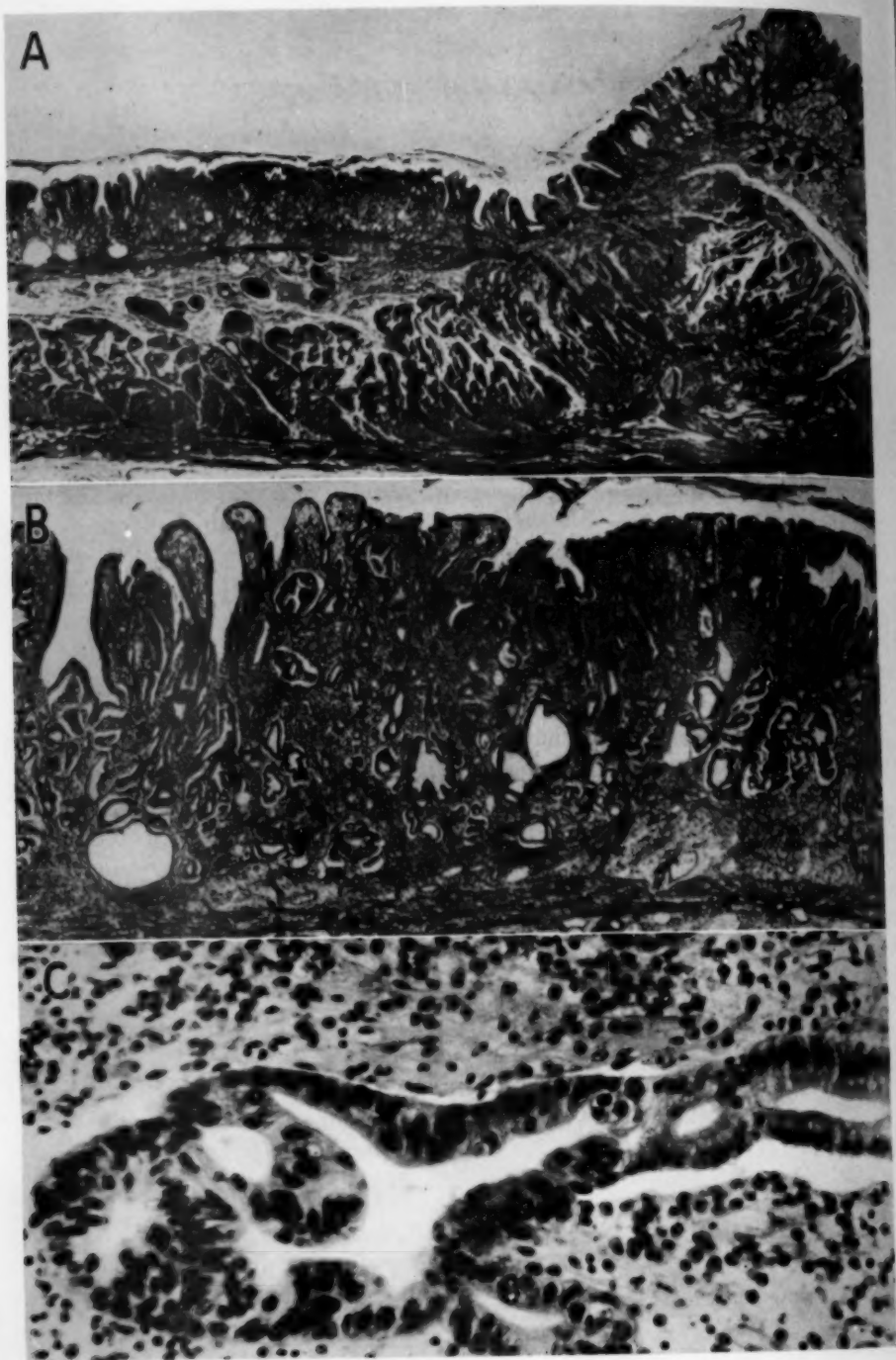


Fig. 1.—*A*, carcinoma in situ of the prepyloric region, filling the site of an ulcer visualized in a roentgenogram three months before (case 1); $\times 10$.

B, portion of the involved area; $\times 50$. Atypical glands with papillary infolding can be seen. The mucosal architecture, though distorted, is maintained, and there is no invasion.

C, detail of a single gland from the same specimen; $\times 350$. To the left the neoplastic cells are hyperchromatic and the nuclei large and disoriented. The sudden transition to normal cells is evident.

cinoma *in situ* had not been developed, they have been variously interpreted. In the last seven years 4 examples of this condition have been seen in this laboratory and 3 noninvasive polyps with malignant cytologic features.

The histologic characteristics of these lesions can best be appreciated by reference to the illustrations. Over an appreciable distance (from 1 to 2.5 cm.) the mucosa, though apparently intact and of normal thickness (fig. 1 *A*), is evidently abnormal when closely inspected. The architecture of the glands may be perfectly maintained or may present minor abnormalities, such as papillary infolding of the epithelium, slightly increased branching or even secondary gland formation (fig. 1 *B*). The striking change, however, is not in the architecture of the glands but in the character of the cells which line them. These cells are usually hyperchromatic, both nuclei and cytoplasm staining more intensely than normal (figs. 1 *C* and 2 *A*). The nuclei are large both absolutely and relatively in proportion to the size of the cell. Nucleoli are also big and stain intensely. Mitotic figures, which are normally sharply restricted to the so-called neck zone of the gastric glands, are numerous and are found in equal numbers at all levels of the mucosa, including the superficial epithelium between the mouths of the glands. Multipolar mitoses are not infrequent (fig. 2 *B*). Most striking of all is the disorientation of the nuclei, suggesting complete loss of polarity in the cells. Under normal conditions—and even severe gastritis produces little change in this respect—the nucleus of the gastric epithelial cell is uniformly oriented at the base of the cell, closely adjacent to the stroma. The only exception to this is in the neck zone, where a nucleus about to undergo mitosis wanders up to the apex of the cell before dividing, a phenomenon identical with that seen in other glandular tissues—for instance, in the proliferating endometrium. Even in this neck zone, however, the majority of the nuclei will be basal; a few, usually obviously in some stage of mitosis, will be apical, and very few will be seen in intermediate positions. In carcinoma *in situ*, in contrast, there is frequently almost complete disorientation, and nuclei are found with equal frequency at all levels in the cells. Since the cells are often somewhat elongated, this may produce a false impression of a multi-layered epithelium (fig. 2 *A*).

A phenomenon that is very interesting is that this cytologic alteration frequently does not involve the entire gland. The interglandular surfaces and the neck zones are regularly affected, but the process extends downward to a variable depth. Frequently it reaches to the bottom of the gland, but often, even in the center of the gross area of involvement, the basal section of many glands will appear quite normal. Less obvious, but usually to be found with careful examination, will be small

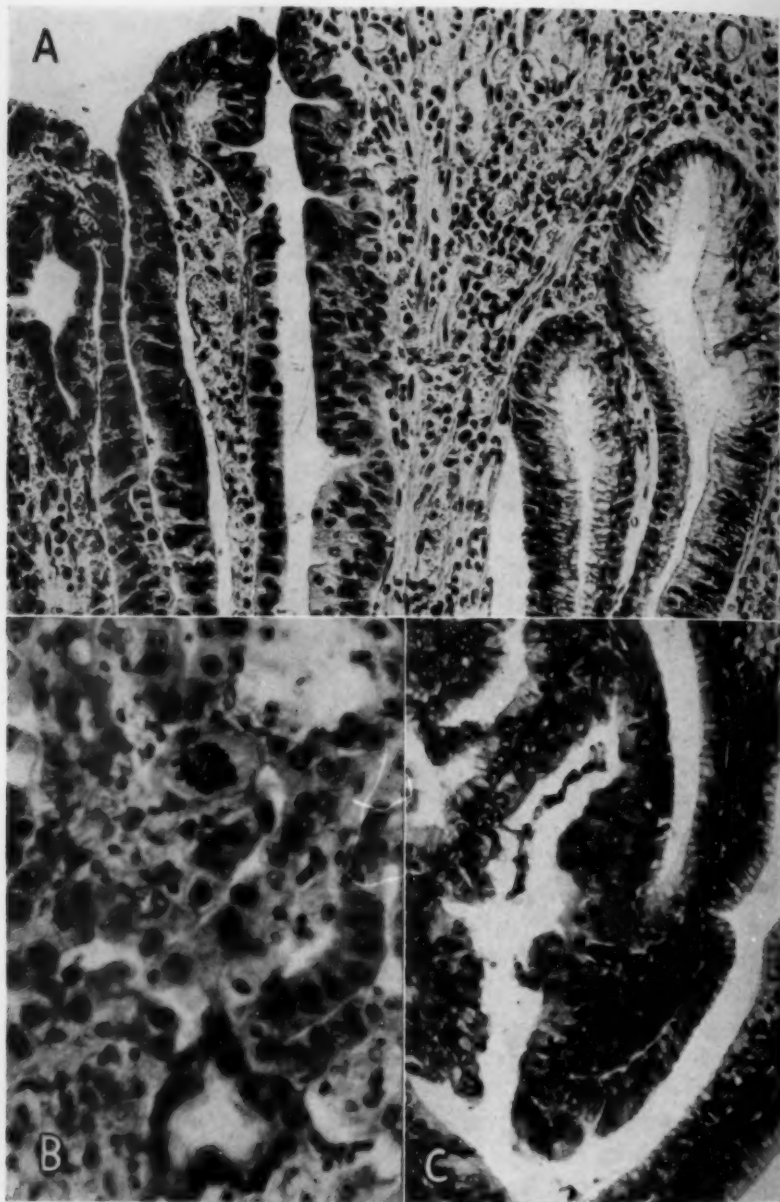


Fig. 2.—*A*, carcinoma in situ from a prepyloric ulcer; $\times 179$. On the left, neoplastic cells line the tubules, showing hyperchromatism, nuclear disorientation and gigantism, and pseudostratification. The two glands on the right are normal. *B*, the same carcinoma showing multipolar mitosis; $\times 358$. *C*, detail from a non-invasive, cytologically malignant polyp, showing the sudden transition from neoplastic to normal cells; $\times 89.5$.

stretches of six to twenty normal cells, even in the superficial epithelium or in the mouths of the glands, which have apparently resisted replacement though completely surrounded by the tumor. Islands of intestinal metaplasia appear particularly resistant and frequently persist in numerous foci within the neoplastic zone. Where ulceration or erosion has occurred, regeneration from such persistent normal cells may occur in a significant degree.

Equally interesting is the behavior at the margin of the area of involvement. In serial sections this can be studied at many spots, and the process is always the same. One gland will show neoplastic epithelium; the next will be absolutely normal (fig. 2 A). In fortunate sections one may be able to observe a single gland with neoplastic epithelium on the right side and normal epithelium on the left. Occasionally, indeed, one catches the spot where one cell can be clearly recognized as cancer, and the next cell, as normal (fig. 1 C).

This sharpness of border is, in fact, an important diagnostic sign. On the border of a healing erosion or ulcer, rapidly regenerating epithelium is frequently found. This may be moderately hyperchromatic and may lack polarity, and mitotic figures will, of course, be numerous. However, as one passes back from the proliferating margin the degree of hyperchromatism diminishes fairly rapidly but also gradually, and as polarity reappears nuclei become more basal and signs of cytoplasmic differentiation, such as mucin secretion, appear. Such transition stages are entirely lacking in carcinoma in situ.

What collateral evidence can be offered beyond the purely cytologic arguments that these lesions are cancerous? First, for what it is worth, is the argument of location. The marked preponderance of malignant lesions in the prepyloric area has long been known (Orator¹⁰). Hampton's estimate of the frequency of carcinoma versus that of benign peptic ulceration in the "pre-pyloric" area (12 to 1) was unquestionably too high. If, however, his definition of the prepyloric area (the last inch of the stomach before but not including the pylorus) is adhered to, the preponderance of malignancy is certainly at least 3 to 1, as Sampson and Sosman¹¹ recently showed in a large series of cases. The lesions which we have described and illustrated came from this segment of the stomach which is particularly prone to suffer from malignant rather than benign ulcers.

Secondly, a single case has been observed in which an extensive carcinoma in situ presented minimal, presumably incipient invasion. A large area of intermittent shallow erosion was present which was bounded on either margin by typical carcinoma in situ, with persistent

10. Orator, V.: *Virchows Arch. f. path. Anat.* **256**:202, 1925.

11. Sampson, D. A., and Sosman, M. C.: *Am. J. Roentgenol.* **42**:797, 1939.

foci of carcinomatous mucosa between the zones of erosion and with a single spot of invasion, only 2 mm. in diameter, into the submucosa. It seems fair to interpret this lesion as one caught virtually at the point of transition from carcinoma in situ to invasive cancer and therefore to cite it as evidence that such lesions do in time become invasive. An identical case was reported and beautifully illustrated by Moszkowicz.⁸

A third line of evidence may be drawn from a study of gastric polyps. Their pronounced tendency toward "malignant degeneration" is generally admitted. Among these a significant number are found in which all the features of carcinoma in situ are perfectly reproduced. The maintenance of normal, albeit exaggerated, glandular architecture and the cytologic atypicality, characterized by loss of polarity, hyperchromatism, nuclear and nucleolar gigantism and excessive numbers of mitotic figures, are identical. Similar also is the presence of a few unaltered deep glands at the base of the lesion. Noteworthy is the sharp margin—one gland is neoplastic, the next one clearly normal, with no hint of transition zone (fig. 2 C). From the edges of such a polyp, moreover, nonpolypoid carcinoma in situ may spread, replacing the normal epithelium for a distance of 1 cm. or more (fig. 3 A).

Finally, established carcinoma may on occasion extend for significant distances along the mucous membrane as carcinoma in situ. This must be distinguished sharply from lymphatic permeation and direct invasion of the mucosal tissue spaces, a favorite method of spread in signet ring carcinoma. In true carcinoma in situ extension is directly by continuity along the epithelial surfaces, with rigid respect for basement membranes. Under these conditions, too, the deeper portions of the glands are frequently uninvolved, and it is characteristic that islands of intestinal metaplasia usually resist replacement, and the tumor tends to encircle without involving them (fig. 3 B).

In summary, then, lesions of the stomach have been described which present the usual cytologic criteria now recognized as characteristic of the noninvasive stage of carcinoma, so-called carcinoma in situ. As collateral evidence for their malignant character may be cited their frequent location in the prepyloric zone, the demonstration of incipient invasion in a case, the identity of the cytologic changes with those of certain noninvasive gastric polyps, the extension of the malignant cytologic process as typical carcinoma in situ from such polyps over adjacent nonpolypoid portions of the mucosa, and finally the spread of established invasive carcinoma in similar fashion over significant distances in the mucosa.

The clinical importance of recognition of such a stage in carcinomatous development is obvious. Only with invasion of lymphatics and

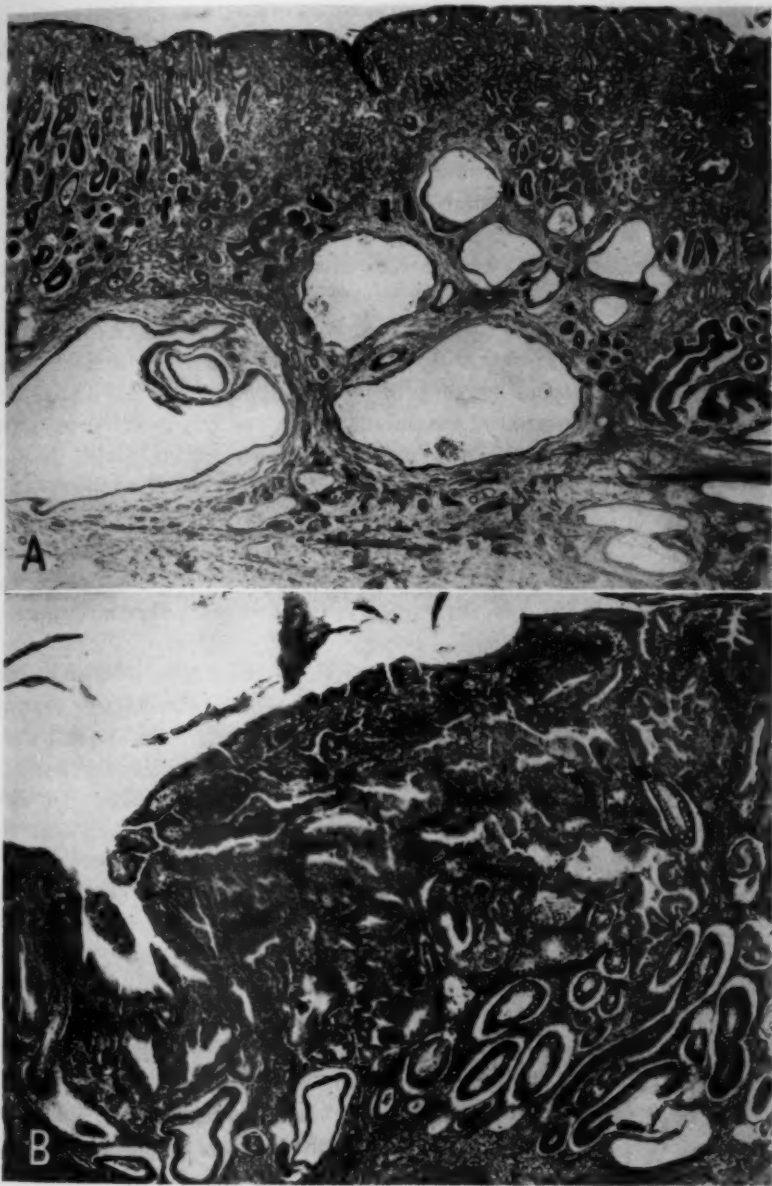


Fig. 3.—*A*, extension of malignant cytologic structure from a polyp as carcinoma in situ; $\times 21$. *B*, extension as carcinoma in situ of established carcinoma; $\times 42.5$. The deeper glands of the mucosa show intestinal metaplasia but are uninvolved by tumor.

blood vessels does metastasis become a probability, and therefore true carcinoma in situ should be completely curable by surgical excision. In the group of cases under discussion there has been no evidence of recurrence or metastasis in the intervals from one to seven years since resection.

II

The majority of gastrointestinal cancers undergo, sooner or later, ulceration varying from minor superficial erosion to deep crater formation, which may on occasion result in perforation of the viscus. It is ordinarily assumed that two factors are chiefly responsible: infection and imperfect blood supply. In regard to the stomach another possibility must be considered. If free acid is present, and in the early stages of the disease it not infrequently is, the infectious element is likely to be minimized, but the possibility of "peptic" ulceration exists. The probability of peptic ulceration of carcinomatous tissue has received comparatively little attention in this country but has been given detailed consideration in Germany by Gruber,¹² Stromeyer,⁹ Borrmann⁷ and others.

In an ulcerated lesion of the stomach is it possible by histologic means to distinguish peptic ulceration? There is reason to believe that in the active, progressive stages of peptic ulceration this is frequently feasible.

In a concurrent study of benign peptic ulceration in this laboratory, to be published shortly by John I. Bradley, several characteristic features of benign peptic ulceration have been pointed out. The typical acute progressive ulcer will show a loss of substance, an inner zone of necrosis, somewhat resembling postmortem digestion (though present in freshly resected lesions), composed of chromophobic, shadow-like cell outlines; bordering this, a conspicuous, brilliantly acidophilic layer, usually described as fibrinoid necrosis, then often a layer of true fibrin and intense leukocytic infiltration. A most characteristic feature is the sharp crescentic line of necrosis in the emigrating leukocytes. At a uniform distance from the margin of the ulcer they suddenly become necrotic but can still be recognized as shadow forms within the zone of fibrinoid. In chronic ulcers any or all of these layers may be missing, but if the ulcer is active the "fibrinoid" eosinophilic zone is constant. With subsidence of the acute stage the fibrinoid layer becomes infiltrated with leukocytes, honeycombed, and is eventually digested away, absorbed or organized. The final picture is not histologically distinctive.

It should be emphasized that no single feature of this reaction is pathognomonic. "Fibrinoid" is of course quite nonspecific, and similar homogeneous acidophilic exudates are found in a great variety of inflam-

12. Gruber, G. B.: *Ztschr. f. Krebsforsch.* **13**:105, 1913.

matory lesions. The total picture of the acute progressive stage is, however, reasonably specific: the sharply punched-out loss of substance, the clearly defined crescentic layers of the ulcer wall, and their uniform arrangement and thickness throughout the extent of the lesion.

Ulcerations presenting many of these characteristics are occasionally found in established gastric carcinomas. Figure 4*A* illustrates such a case. It has been photographed with a green filter, so that the brilliantly acidophilic zone appears nearly black. Its sharp demarcation and its uniform thickness are typical. With higher power it is evident that the luminal portion is completely necrotic. Leukocytes penetrate its outer layers from the underlying living tissue, but at a uniform distance from the lumen they suddenly become necrotic and can be recognized only as shadow forms in the innermost layer. Yet this ulcer is completely surrounded by, and, since it is fairly acute, must have developed within, carcinomatous tissue.

In instances such as that shown in figure 4*B* the ulceration has passed completely through the tumor down to the subserosal fat tissue. The reaction is precisely similar at the base, however, where its peptic character cannot be doubted, and on its lateral margins, where it is bordered by neoplastic tissue.

Ulceration of this type is not the rule in gastric carcinomas. In the last two years it has been noted only ten times among 93 resected carcinomas. In all of these 10 cases but 1, in which symptoms of pyloric obstruction dominated the clinical picture, there was a history of periodic pain relieved by food or soda, and in all the clinical diagnosis of benign ulcer was made or seriously considered. In 6 of the 10 cases gastric analyses were made, and all showed free hydrochloric acid following administration of alcohol or histamine. In 2 cases there was frank hyperchlorhydria. Of the remaining 4 cases, in which gastric analyses were not made, there was in 1 a coincidental duodenal ulcer (proved by roentgenogram and surgical exploration), and in a second, a benign gastric ulcer on the side of the stomach opposite from the ulcerated carcinoma. The presence of free acid can reasonably be inferred in these 2 cases as well. Within this period no similar histologic picture has been met in any case with demonstrated anacidity.

Confirmatory evidence for the peptic character of the ulceration associated with certain gastric cancers is provided by the following roentgenologic observations, reviewed and verified by Dr. Richard Schatzki.

CASE 1.—A high-strung, emotionally unstable married woman of 48 had suffered for eight years from indefinite gastric symptoms, though roentgenologic examinations showed a normal gastrointestinal tract. One month before her entry into the hospital persistent epigastric discomfort appeared, and new roentgenologic examinations showed a persistent ulcer crater in the prepyloric region. Gastric aspiration showed free hydrochloric acid. Surgical treatment was advised, but the

surgeon on exploration found so little visual or tactual abnormality that he felt resection was not justified. The patient was placed on a strict medical regimen for ulcer. Two months later, roentgenologic examination showed disappearance of the crater but persistence of abnormality, evidenced by absence of normal rugae and interference with the peristaltic waves. Surgical operation was again advised, and a resection of the pyloric portion of the stomach was carried out despite very slight and equivocal gross evidence of abnormality.

The resected specimen showed, 2 cm. from the pylorus, an irregular smooth area in the gastric mucosa, 2 cm. in diameter, very slightly elevated but not indurated or ulcerated. Histologic examination showed replacement of the normal mucosa over the area of involvement by carcinoma *in situ* (fig. 1). Though points of superficial erosion were present, there was no trace of the previous ulcer crater.

CASE 2.—A Polish textile worker of 53 had been troubled by epigastric distress and occasional vomiting following meals for three years. His symptoms grew progressively worse and he lost weight and strength. Two weeks before his entry into the hospital he began to vomit everything he ate. Gastric analysis showed free hydrochloric acid. Roentgenologic examination showed two ulcer craters: one, 1.5 cm. in diameter, on the lesser curvature, just below the angle of the stomach; the second and much larger one, 3 by 1.5 cm., in the immediate prepyloric area. There was considerable stasis. It was felt that the smaller ulcer was certainly benign, the larger one probably so. Medical treatment was advised and instituted. Reexamination sixteen days later showed that the smaller ulcer had almost disappeared and that the larger one had markedly decreased in size. Because of persistent pyloric obstruction and the possibility of malignancy, surgical operation was decided on and a partial gastrectomy performed.

The resected stomach showed four separate erosive lesions of varying depth along the lesser curvature, all of which proved perfectly benign on microscopic examination and showed no areas of active peptic ulceration but well marked healing and regeneration. Directly in the pylorus was a deep, sharply punched-out ulcer, 1.5 cm. in diameter. Microscopic examination showed persistent active erosion in part of the ulcer bed, regenerative activity on the margin toward the duodenum and invasive signet-ring carcinoma on the antral side of the ulcer.

CASE 3.—An Italian laborer of 44 noted the onset of crampy epigastric pain, not related to the intake of food, eight months before admission. Soda afforded fairly prompt relief. Three weeks before his entry into the hospital the stools became black, and on the day before admission he vomited red blood. The vomitus showed free hydrochloric acid. Roentgenologic examination showed a deep ulcer crater, 1 cm. in diameter, lying directly in the pyloric valve on the side of the lesser curvature of the stomach. It was interpreted as benign. After twenty days of medical therapy the crater was still present but only 0.5 cm. in diameter. The man was discharged nearly symptom free, but vomiting recurred immediately, and he was readmitted one week later. Because of persisting obstruction, surgical operation was advised and a subtotal gastrectomy performed.

The specimen showed a deeply punched-out ulcer, 1 cm. in diameter and 1.5 cm. in depth. Its walls felt hard, and one lymph node showed obvious metastasis. Microscopic examination showed signet ring car-

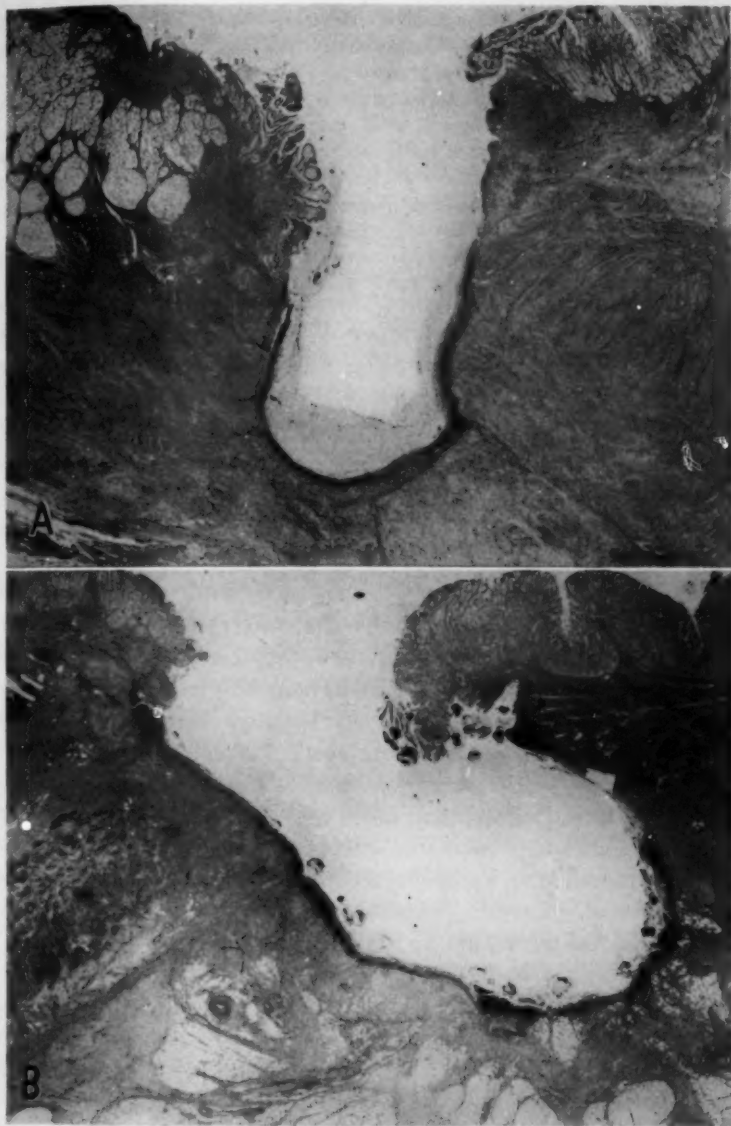


Fig. 4.—*A*, deep peptic ulceration of a signet ring carcinoma (case 3); $\times 6$. The ulcer is completely surrounded by cancerous tissue. Its walls show the typical crescentic layers of peptic ulceration. *B*, deep peptic ulceration through a signet ring carcinoma into non-neoplastic fat tissue; $\times 5$. The walls of the ulcer show a similar reaction in neoplastic and non-neoplastic areas.

cinoma in the walls and underlying the entire ulcer crater. The walls of the latter showed the typical features of active peptic ulceration described in the text (fig. 4 A).

CASE 4.—An Irish iron worker of 62 entered with a story of indigestion and heart burn of two years' duration. His symptoms showed no definite relationship to meals but were relieved by soda. Gastric analysis showed free hydrochloric acid. Roentgenologic examination showed an ulcer crater, 2.5 cm. in diameter, on the lesser curvature. It was surrounded by a large area of induration. The roentgenologic diagnosis was benign ulcer, but Dr. E. B. Benedict on gastroscopic inspection of the lesion suspected malignancy. After two weeks of medical treatment reexamination by roentgen ray showed reduction both in the size of the crater and in the area of induration by about 50 per cent. Repeated roentgenologic examinations showed little change for the next eight months; then the crater began rapidly to enlarge again. Surgical exploration revealed a large chronic ulcer of the lesser curvature and posterior wall, and a resection was performed.

The specimen showed an ulcer of the posterior wall adjoining the lesser curvature, measuring 3 by 3.5 cm., which was 1 cm. in depth. Around the ulcer margins the mucosa was reddened for a distance of 1 to 2 cm. Microscopic examination showed typical chronic but active peptic ulceration with a base of dense scar tissue. The bordering mucosa for a distance of 2 to 10 mm. was replaced by carcinoma *in situ*.

COMMENT

The objective roentgenographic evidence of diminution in size of the ulcer craters in these 4 cases under the influence of medical therapy constitutes strong evidence in favor of the peptic character of the ulceration. In each instance, on gastric aspiration the presence of free hydrochloric acid was demonstrated. On subsequent examination 3 of the lesions showed the histologic picture which I have described as characteristic of active peptic ulceration and 1 specimen showed multiple simultaneous benign ulcers and erosions. In case 1 the ulcer crater disappeared completely under the effects of treatment. The functional evidence is therefore in complete accord with the histologic that these ulcerations were, in truth, "peptic," and the observations serve as a valuable check on the reliability of the histologic criteria.

In summary, histologic patterns of ulceration identical with those of active benign peptic ulceration have been described in a group of gastric carcinomas. These comprised approximately 10 per cent of the malignant specimens which passed through the laboratory in a two year period. The symptoms in 9 of the cases showed the typical relationship to food intake of peptic ulceration. In 8 the presence of free hydrochloric acid either was demonstrated or might safely be assumed from the simultaneous presence of separate foci of benign peptic ulceration. Roentgenologic evidence of partial healing of the ulcer crater

under the effects of medical regimens of ulcer therapy was demonstrated in 4 patients in whom the lesions were subsequently proved by resection to be carcinomatous. Three of these lesions showed the typical histologic patterns which I have described as characteristic of peptic ulceration; in the third the area of the former ulcer crater was completely filled with carcinoma in situ. Histologic and clinical evidence and response to therapy all agree in pointing toward the peptic character of the ulcerations within these tumors.

RELATIONSHIP OF PEPTIC ULCERATION TO CARCINOMA

Few points in pathology have been more bitterly debated with less result than the subject of the relationship of peptic ulceration and carcinoma of the stomach. Estimations of the frequency with which ulcers undergo malignant change vary from 1 per cent (Borrmann⁷) to 71 per cent (Wilson and MacCarty¹³). An excellent review of the literature has been compiled by Borrmann, and it would be pointless to repeat it. It may be roughly summarized as follows: Clinical statistics for the most part agree that chronic gastric ulcers rarely become carcinomatous. Conversely, statistics compiled from gross pathologic study indicate that few cancers arise from ulcers since benign ulcers are almost limited to the lesser curvature and the pyloric ring whereas cancers most frequently arise in the prepyloric area and other parts of the stomach. From these facts it is clear that most ulcers do not become carcinomatous and few cancers are preceded by peptic ulceration.

The arguments in favor of the development of cancer in ulcer are primarily histologic. They rest on the finding of small foci of neoplasm in the walls of an ulcer which, to judge from size and the degree of scar formation in its base, must be of some degree of chronicity. Because the focus of recognizable neoplasm is small, it is assumed that it must be of very recent origin and that the ulcer antedates it. Reasonable as such a conclusion seems at first glance, we believe that the facts presented in this paper throw some doubt on its validity.

Carcinoma in situ occurs, as has been pointed out, in numerous organs. In those which are readily accessible to examination, such as the skin, the nipple and the cervix uteri, it has been possible to watch these tumors over long periods of time, and it has been proved that the tumor may remain "in situ" for periods of months to years. Though observations of this type on the stomach are naturally lacking, it is not unreasonable to infer a similar situation, that here, too, carcinoma may exist for long periods of time in a preinvasive state. Therefore limitation of tumor to the mucosa does not mean that it is necessarily of recent origin.

13. Wilson, L. B., and MacCarty, W. C.: *Am. J. M. Sc.* **165**:846, 1909.

In part II it was demonstrated that peptic ulceration of carcinomatous tissue occurs. It is logical to assume that this would be most frequent in the early stages of carcinomatous involvement of the stomach, when secretion is still fairly normal. If peptic ulceration occurred in an area of carcinoma *in situ*, it would quickly ulcerate through the shallow layer of neoplastic tissue, most of which would be destroyed. Though theoretically the entire tumor might ulcerate away, as Stromeier has suggested, this seems improbable. However, a deep peptic ulcer might develop with only fragments of persisting tumor about its margins. Such a picture has usually been interpreted as evidence of ulcer developing in cancer. But if carcinoma *in situ* behaves in the stomach as it does in other organs, the cancer may well have developed before the ulcer. One of the major arguments for the development of carcinoma in ulcer is therefore open to question, if not invalidated.

It is evident that the greatest caution is necessary in attempting to interpret from the histologic picture the genesis of a malignant ulcer. Lack of such conservatism is all too evident in much that has been published on the subject and accounts in large measure for the extraordinary variation in the conclusions which have been reached. Proof of the sequential relationship of ulceration and neoplastic development is extremely difficult in the individual case. Certainly no single criterion is sufficient, and clinical, roentgenologic and pathologic evidence in combination may still prove inadequate. Interpretive analyses of individual cases, especially those based on purely histologic criteria, should not be permitted to weigh unduly against conservative statistical analyses.

SUMMARY

The noninvasive stage of cancer of the stomach has been described and illustrated on the basis of 4 cases in which nonpolypoid lesions were associated with peptic ulceration. Histologic evidence has been presented that secondary peptic ulceration of cancerous tissue in the stomach is not uncommon, and the validity of the histologic criteria has been confirmed by roentgenologic evidence of diminution in the size of the ulcer crater under medical treatment in 4 cases. The significance of these findings in relation to the genesis of the "malignant ulcer" has been discussed.

MECHANISM OF LEUKOCYTOSIS WITH INFLAMMATION

THE NATURE OF THE LEUKOCYTOSIS-PROMOTING FACTOR IN EXUDATES

VALY MENKIN, M.D.

BOSTON

Studies of the various mechanisms concerned with the development of the inflammatory reaction¹ have led to the recent isolation of a nitrogenous substance, termed leukotaxine, from exudates which is per se capable of reproducing two of the basic sequences of inflammation, namely, the initial increased permeability of capillaries and the migration of polymorphonuclear leukocytes.² Leukotaxine injected into the blood stream or subcutaneously in either dogs or rabbits fails to alter the number of circulating leukocytes.³ The leukocytosis frequently associated with inflammatory processes has been found to be evidently referable to the presence in exudates of a leukocytosis-promoting factor.⁴ Hence it seems clear that the migration of leukocytes to the point of injury is scarcely due to the same mechanism which is responsible for the accompanying rise in the white blood cell level. Contrary to the properties of leukotaxine, the leukocytosis-promoting factor is thermolabile and essentially indiffusible through a cellophane membrane. The action of this factor seems to be on the bone marrow, causing a discharge of relatively immature leukocytes into the circulating blood stream.³ Preliminary experiments have indicated that the leukocytosis-promoting factor fails to exert precisely the same type of effect on leukocytes as is elicited by yeast nucleic acid, pentnucleotides, adenosin or histamine.⁵ Some

The investigation was aided by grants from the Milton Fund of Harvard University, the International Cancer Foundation and the Dazian Foundation for Medical Research.

This communication represents paper XIX of a series entitled "Studies on Inflammation," presented before the American Association of Pathologists and Bacteriologists, Pittsburgh, March 22, 1940.

1. Menkin, V.: *Physiol. Rev.* **18**:366, 1938; *Dynamics of Inflammation*, New York, The Macmillan Company, 1940.

2. Menkin, V.: *J. Exper. Med.* **67**:129 and 145, 1938.

3. (a) Menkin, V.: *Science* **90**:237, 1939; (b) *Am. J. Path.* **16**:13, 1940; (c) *Science* **91**:320, 1940.

4. The earlier literature on the subject has been reviewed elsewhere^{2b} and will therefore not be taken up in the present communication.

5. Menkin, V.: *Am. J. Path.* **10**:193, 1934. Menkin, V., and Warner, C. R.: *ibid.* **13**:25, 1937.

of the properties of this factor have suggested the possibility that it may be a protein or perhaps a product of protein catabolism.^{3b} The present observations on protein fractionation of exudates support the view that the leukocytosis-promoting factor is a globulin or is in close association with the globulin fraction of exudate.

TABLE 1.—*Variation in White Cell Counts on Blood of Normal Dogs*

Dog	Lowest Number of Leukocytes per Cubic Millimeter Within a Period of About Six Hours	Highest Number of Leukocytes per Cubic Millimeter Within a Period of About Six Hours	Percent Increase in Leukocytes
4-74.....	12,850	18,300	42.4
4-78.....	11,150	15,200	36.3
4-71.....	11,000	14,850	35.0
4-71.....	12,100	15,800	30.6
4-73.....	11,650	15,000	28.8
4-73.....	15,400	19,550	26.9
4-73.....	13,600	16,800	23.5
4-71.....	11,500	13,850	20.4
4-78.....	18,200	21,050	15.7
4-79.....	13,850	14,450	4.3
4-73*.....	16,250	20,100	23.7
Average.....	13,395	16,786	26.2

* The counts were made after an intravascular injection of 30 cc. of Sørensen phosphate buffer at *pu* 7.0. In all the aforementioned experiments counts were taken about every hour.

TABLE 2.—*Effect of Exudates on Leukocyte Counts*

Dog	Absolute Number of Leukocytes per Cubic Millimeter Prior to Injection of Material	Highest Level of Leukocytes per Cubic Millimeter Within About Six Hours After Injection of Material	Percent Increase in Leukocytes
4-82.....	6,650	15,050	126.3
4-71.....	12,700	27,250	96.9
4-71.....	13,500	22,250	64.8
4-79.....	26,060	41,250	58.3
4-79.....	16,800	25,150	49.7
4-65*.....	19,350	32,000	65.4
Average.....	16,008	27,158	77.2

* This sample of exudate had been dialyzed against water.

EXPERIMENTS

Samples of peripheral blood were taken from a series of dogs every hour in order to ascertain the normal range of variation in the number of circulating leukocytes during a period of about six hours. The results are summarized in table 1. The maximum increase in counts averaged 26.2 per cent. Studies on an earlier series of animals yielded similar results, i. e., an average increase of 23.8 per cent.^{3b}

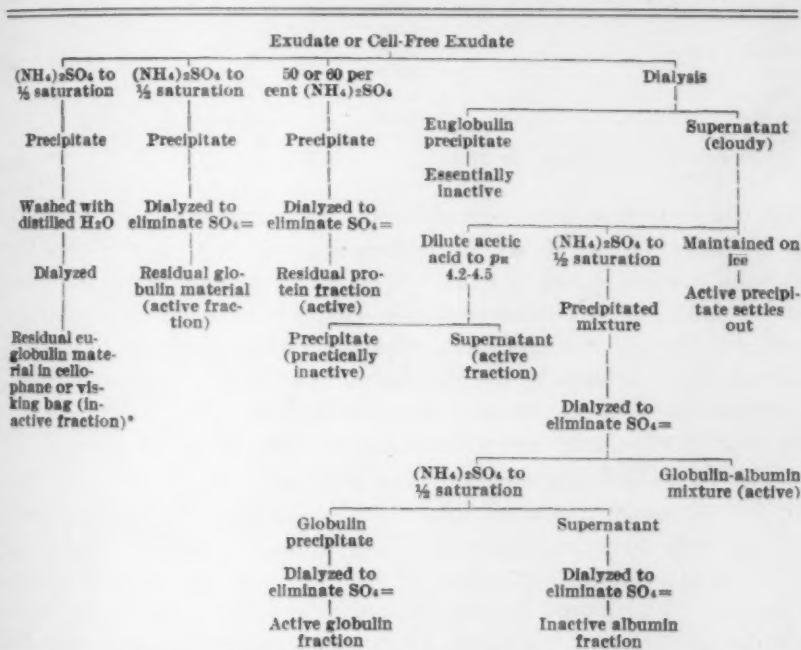
The leukocytosis-promoting factor was found in exudates elicited by a variety of unrelated irritants, e. g., a physical agent, turpentine and croton oil.³ Its presence is demonstrable in a dog's exudate irrespective of the type of irritant

employed to induce the inflammatory reaction. For this reason, in the present series of experiments, in which dogs were used, exudates were obtained solely by the intrapleural injection of about 1.5 cc. of turpentine. This method yielded, as a rule, a considerable amount of exudative material.⁵ A 20 to 30 cc. sample of such an exudate was injected by intracardiac puncture into the circulating blood stream of a normal dog. Earlier experiments had shown that this was satisfactory as a method of inoculation.^{3b} Leukocyte counts on samples of blood obtained from the ear were recorded at intervals of approximately one hour. Exudates were utilized only from donors in which leukocytosis developed as a result of the intrapleural injection of turpentine. The results of several such experiments are summarized in table 2. It is clear that in such exudates there is present a factor which when injected into normal dogs markedly increases the number of circulating leukocytes. The present average increase of 77.2 per cent fully confirms the results obtained in a larger number of published experiments, in which an augmentation averaging 70 per cent has been reported.³

PROTEIN FRACTIONATION OF INFLAMMATORY EXUDATES

In an endeavor to identify the leukocytosis-promoting factor in exudates, the material was analyzed by several different methods. The general scheme utilized is summarized in table 3. The main features may be listed as follows:

TABLE 3.—Scheme of Extraction of Leukocytosis-Promoting Factor



* This fraction is inactive provided the pH of the original exudative material is not markedly acid (i. e., pH 6.5 or below).

1. A sample of about 30 to 50 cc. of exudate contained in a cellophane bag was dialyzed for about one day against running tap water. A precipitate, probably the euglobulin fraction, separated out. This material was centrifuged, taken up in about 20 to 30 cc. of phosphate buffer at either p_H 7.0 or p_H 7.4, and then injected intracardially in a dog. Leukocyte counts were made at approximately hourly intervals. The results, shown in table 4, indicate that in all instances except one the euglobulin fraction obtained by dialysis failed to raise appreciably the level of circulating leukocytes, the rise for all experiments averaging 29.1 per cent. This value is practically of the same magnitude as is encountered in the range of normal fluctuation (table 1). Chick⁶ pointed out a number of years ago that after prolonged dialysis a certain amount of pseudoglobulin is converted into euglobulin. It is possible that in dog 4-72 the signifi-

TABLE 4.—*Effect of the Euglobulin Fraction* of Inflammatory Exudate on Leukocyte Counts*

Dog	Absolute Number of Leukocytes per Cubic Millimeter Prior to Injection of Material	Highest Level of Leukocytes per Cubic Millimeter Within About Six Hours After Injection of Material	Percent Increase or Decrease in Leukocytes
4-72.....	18,500	32,550	76.0
4-73.....	16,150	22,750	40.9
4-71.....	13,350	18,350	37.5
4-73.....	20,400	27,850	36.5
4-71.....	12,000	12,300	2.5
4-78.....	18,600	15,100	-19.0
Average.....	16,500	21,483	29.1

* This fraction was obtained by dialysis.

cant rise in the leukocyte count of 76 per cent is referable to spontaneous precipitation of the leukocytosis-promoting factor from the pseudoglobulin fraction during dialysis. It is, however, also conceivable that in this particular experiment improper separation of the protein fractions occurred during centrifugation, thus accounting for the significant biologic effect induced by the euglobulin. The results of the remaining experiments, however, indicate that the euglobulins of exudates leave the level of circulating leukocytes essentially unaltered.

2. The residual supernatant fraction after separation of the euglobulin by dialysis usually appeared cloudy. This material markedly enhanced the leukocyte count, as shown in the results listed in table 5 (chart 2). This fraction presumably contained both the pseudoglobulin and the albumin. The average increase in leukocyte counts is 64.4 per cent, which approximates fairly closely the effect elicited by the whole exudate (table 2). The results when the albumin fraction was removed suggest

6. Chick, H.: *Biochem. J.* 8:404, 1914.

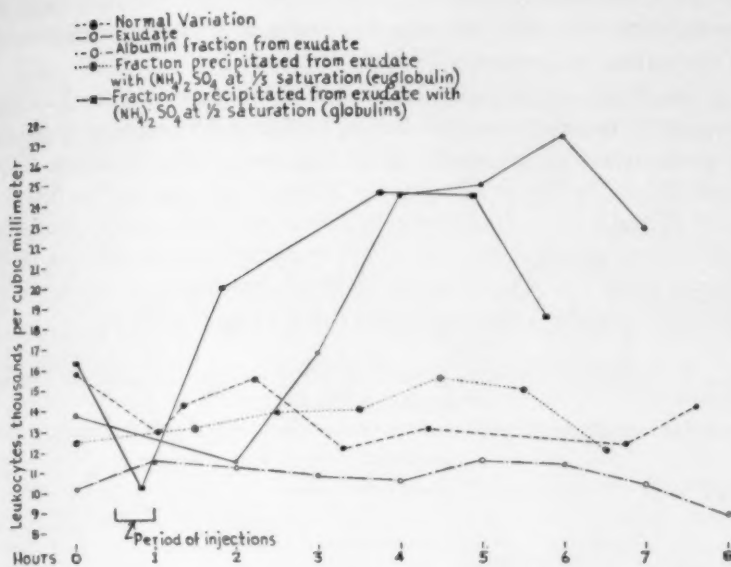


Chart 1.—The effect of an exudate and of several fractions of exudative material, injected into the circulation, on the leukocyte counts of a dog (4-71). The respective fractions utilized and the time of their injection are indicated on the chart.

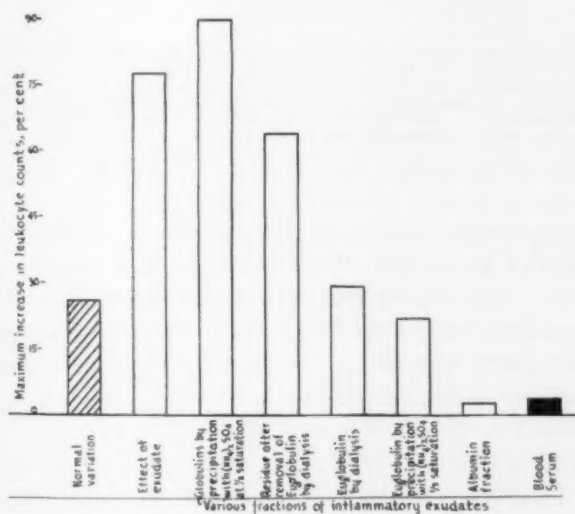


Chart 2.—A diagrammatic representation summarizing the average effects of exudates, of blood serum (see Menkin^{3b} for figures on the effect of blood serum on the number of circulating leukocytes) and of various fractions of exudates on the level of white blood cells. The normal range of fluctuation in the leukocyte counts of several dogs is shown in the first column.

that the leukocytosis-promoting factor is associated primarily with the pseudoglobulin fraction (see dog 4-73, table 5, showing an elevation in the circulating leukocytes of 97.5 per cent).

3. Furthermore, the preliminary addition of acetic acid to the residual supernatant material, to adjust the p_H to 4.2 or 4.5 presumably induced the precipitation of nucleoproteins. The latter were, however, found essentially ineffective in altering the white count (see tables 3 and 5, dogs 4-73 and 4-71). It is also to be noted that if the cloudy supernatant fraction obtained after removal of the euglobulin is placed in a refrigerator at about 3 or 4 C. for a day or two, a precipitate settles out which per se contains the leukocytosis-promoting factor (table 3).

TABLE 5.—Effect on Leukocyte Counts of the Combined Globulin and Albumin Fractions of Exudates *

Dog	Absolute Number of Leukocytes per Cubic Millimeter Prior to Injection of Material	Highest Number of Leukocytes per Cubic Millimeter Within About Six Hours After Injection of Material	Percent Increase in Leukocytes
4-73†.....	16,050	31,700	97.5
4-71.....	11,950	21,900	83.3
4-71§.....	11,900	20,250	71.6
4-78.....	18,750	27,950	49.1
4-78.....	13,650	19,700	44.3
4-72†.....	24,100	33,850	40.5
Average.....	16,050	25,892	61.4

* These fractions were obtained after preliminary removal of the euglobulin fraction by dialysis of the exudate and subsequent centrifugation (see data in table 4).

† These counts represent the results obtained with the globulin fraction largely freed of the albumin fraction by precipitation at half saturation of $(NH_4)_2SO_4$. In dog 4-73 the nucleoproteins were probably also removed by the preliminary acidification of the exudative material with acetic acid at p_H ranging between 4.2 and 4.5.

§ The nucleoproteins were presumably removed by preliminary precipitation with dilute acetic acid at a p_H between 4.2 and 4.5.

4. The supernatant fraction in question can be purified further by precipitation with ammonium sulfate $([NH_4]_2SO_4)$ at one-half saturation (table 3). The heavy white precipitate as a rule failed, even after prolonged centrifugation, to separate from a clear supernatant phase. The combined cloudy mixture was dialyzed for one or more days against running tap water to eliminate the sulfate ions. The resulting fraction, which contained both globulin and albumin, displayed considerable activity. Further treatment with ammonium sulfate at one-half saturation differentiated the globulin precipitate from the albumin-containing supernatant fraction. The dialyzed globulin fraction induced considerable rise in the number of circulating leukocytes (dogs 4-73 and 4-72, table 5). The albumin, on the other hand, was wholly ineffective;

the results are summarized in table 6. The average increase of several experiments with the albumin fraction was found to be 2.7 per cent.

These facts rule out the euglobulin and the albumin fractions, and therefore strongly suggest that the leukocytosis-promoting factor is in the pseudoglobulin fraction of exudate. This inference is borne out in subsequent experiments presently to be described.

5. A few observations were made on the precipitate obtained by treating the exudate with ammonium sulfate at one-third saturation. At this concentration of the ammonium salt euglobulins are known to be precipitated out. The material was centrifuged and the precipitate dialyzed against running tap water as described in the preceding experiments. The results of three such experiments (table 7) indicate an average maximum rise in the leukocyte counts of 21.3 per cent. These observations further support the belief that the leukocytosis-promoting factor

TABLE 6.—*Effect of the Albumin Fraction from Exudate on Leukocyte Counts*

Dog	Absolute Number of Leukocytes per Cubic Millimeter Prior to Injection of Material	Absolute Number of Leukocytes per Cubic Millimeter Within About Six Hours After Injec- tion of Material	Percentual Increase or Decrease of Leukocytes
4-71.....	10,150	11,550	13.8
4-71.....	12,650	14,050	11.1
4-74.....	21,000	20,350	— 3.1
4-79.....	20,850	18,550	—11.0
Average.....	16,162	16,125	2.7

probably does not separate out to any appreciable extent with the euglobulin fraction of exudate. On the other hand, several observations were made on intrapleural exudates removed from the pleural cavity after two successive injections of turpentine. This drastic method of inducing an inflammatory reaction invariably produced an abundant exudate having an acid reaction.⁵ Samples of 30 to 40 cc. of such exudative material salted out at one-third saturation with ammonium sulfate yielded fractions containing appreciable amounts of the leukocytosis-producing factor. This fact, however, is not wholly surprising when it is recalled that the solubility of plasma proteins in salt solution is dependent on the p_H .⁷ Butler and his associates demonstrated quite clearly that the solubilities of all the fractions of plasma proteins increase with increasing p_H .⁸ It is therefore conceivable that when an exudate at

7. Chick, H., and Martin, C. J.: *Biochem. J.* **7**:380, 1913. Sørensen, S. P. L., and Høyrup, M.: *Compt. rend. d. trav. du lab. Carlsberg, série physiol.* **12**: 213, 1915-1917.

8. Butler, A. M.; Blatt, H., and Southgate, H.: *J. Biol. Chem.* **109**:755, 1935.

an acid reaction is treated with ammonium sulfate for "salting out" purposes a decrease in the solubility of its various fractions may become manifest. Under such circumstances the leukocytosis-promoting factor would be precipitated out from an acid exudate at a reduced concentration of ammonium sulfate.

When an inflammatory exudate was treated with 14 per cent disodium sulfate (Na_2SO_4), the active factor failed to separate from the material. According to various authors,⁹ the euglobulin fraction is salted out at

TABLE 7.—Effect on Leukocyte Counts of Fractionating Exudate with Ammonium Sulfate at Various Concentrations

Dog	Concentration of $(\text{NH}_4)_2\text{SO}_4$	Absolute Number of Leukocytes per Cubic Millimeter Prior to Injection of Material	Highest Number of Leukocytes per Cubic Millimeter Within About Six Hours After Injection of Material	Percentual Increase
4-73	Precipitation with	13,350	21,800	138.2
4-82	$(\text{NH}_4)_2\text{SO}_4$ at one-half saturation	9,700	20,250	108.8
4-78		16,900	32,400	91.7
4-73		15,550	24,250	56.0
4-71		16,350	24,500	49.8
Average.....		14,370	26,640	88.9
4-74	60 per cent.....	12,400	21,050	69.8
4-73	50 per cent.....	20,500	34,050	66.1
4-84	50 per cent.....	17,250	26,900	55.9
4-71	60 per cent.....	12,400	18,250	47.2
4-73*	60 per cent.....	10,500	15,150	44.3
Average.....		14,610	23,080	56.7
4-79	Precipitation with	19,100	24,250	27.0
4-71	$(\text{NH}_4)_2\text{SO}_4$ at one-third saturation†	12,450	15,500	24.5
4-82		15,100	16,950	12.3
Average.....		15,550	18,900	21.3

* The supernatant fraction also evidently contained the active leukocytosis-promoting factor to some extent. It remained cloudy in spite of centrifugation. This cloudy supernatant fraction induced, when injected into dog 4-73, a rise of 43.5 per cent in the leukocyte counts.

† When the reaction of the original exudate was markedly acid, precipitation at one-third saturation with ammonium sulfate yielded a heavy precipitate containing the leukocytosis-promoting factor.

this concentration. This would substantiate further the foregoing observations that when the exudate to begin with is at an alkaline or a neutral stage the leukocytosis-promoting factor fails, as a rule, to separate with the euglobulin fraction. Finally, it is worth while to point out the general fact that when precipitated fractions showed a relative absence of the leukocytosis-promoting factor the active factor was found in the supernatant phase and vice versa.

9. Gille, R.: *Compt. rend. Soc. de biol.* **121**:906, 1936. Peters, J. P., and Van Slyke, D. D.: *Quantitative Clinical Chemistry*, Baltimore, Williams & Wilkins Company, 1932, vol. 2.

6. Observations were made on the effect of higher concentrations of ammonium sulfate on exudates. When the material derived from a donor with definite leukocytosis was salted out at one-half saturation of ammonium sulfate, the precipitate, free of its sulfate ions, invariably induced in normal dogs a marked rise in the number of circulating leukocytes, averaging 88.9 per cent (table 7). The volume of the samples studied ranged from 25 to 50 cc. of exudate. The material was salted out with an equal volume of saturated ammonium sulfate. The results indicate that the leukocytosis-promoting factor is entirely salted out along with the total globulin fraction. Similar samples of exudates treated with either 50 or 60 per cent ammonium sulfate yielded pre-

TABLE 8.—Effect of Various Fractions of Blood Serum on Leukocyte Counts

Dog	Type of Material Injected	Absolute Number of Leukocytes per Cubic Millimeter Prior to Injection of Material	Highest Level of Leukocytes per Cubic Millimeter Within About Six Hours After Injection of Material	Percent Increase or Decrease in Leukocytes
4-71	Euglobin fraction*.....	14,950	14,000	-6.4
4-71	Pseudoglobulin fraction†....	8,900	9,750	+9.6
4-65	Albumin fraction§.....	23,950	26,050	+8.8
4-71	Globulin fraction by precipitation with (NH ₄) ₂ SO ₄ (60%)#.....	11,300	12,150	+7.5
4-65	Albumin fraction with probably some residual globulin fraction¶.....	20,550	19,200	-6.6

* This was obtained by dialysis of blood serum.

† This was the pseudoglobulin obtained after removal of euglobulin from serum by dialysis, followed by precipitation with ammonium sulfate at one-half saturation.

‡ This was the albumin fraction obtained after removal of euglobulin by dialysis and then removal of the pseudoglobulin by precipitation at one-half saturation with ammonium sulfate.

This was the globulin fraction obtained by precipitating serum with an equal volume of 60 per cent ammonium sulfate.

¶ This was the albumin fraction, with a probable admixture of globulins, obtained after partial removal of the globulin fraction by treatment of serum with 60 per cent ammonium sulfate.

cipitates containing the leukocytosis-promoting factor in moderate concentration. The results of several experiments with these fractions, recorded in table 7, indicate an average rise in the level of circulating leukocytes of 56.7 per cent. The biologic effect is thus somewhat less pronounced than that caused by the fraction salted out at one-half saturation with ammonium sulfate. The lower absolute amount of the salt utilized in each volume of sample treated with 50 or 60 per cent of ammonium sulfate readily explains the differences in results.

In an endeavor to determine whether any active fraction of plasma proteins could be obtained from blood serum several experiments were performed on samples obtained from normal dogs. The results and method utilized are summarized in table 8. It is evident that the globulin fractions of blood serum fail to alter the level of circulating leukocytes.

COMMENT

The observations presented substantiate further the earlier studies³ on the presence of a leukocytosis-promoting factor in inflammatory exudates of dogs. The demonstration of this factor is particularly striking in material obtained from donors with leukocytosis accompanying acute inflammation. Previous evidences showed that the factor is primarily indiffusible and thermolabile.^{3b} The present series of experiments indicate either that the leukocytosis-promoting factor is a protein or that it is at least associated with a protein group. The active principle is precipitated out at one-half saturation with ammonium sulfate. The factor is therefore salted out with the globulin fraction. Its inability to be dialyzed and its inactivation at 60 C. support the belief that it is most likely protein in nature. The results on fractionation of the exudative material strongly suggest that it is a globulin. There is little evidence to indicate that the factor is a euglobulin. The fractions obtained after dialyzing the exudate, after precipitation at one-third saturation with ammonium sulfate or after treatment with 14 per cent sodium sulfate support this conclusion. Since the leukocytosis-promoting factor is clearly not an albumin (table 6), the evidences indicate its probable presence in the pseudoglobulin fraction of inflammatory exudate.

The hourly effects on the leukocyte level of several different fractions of exudate administered on separate days to an animal (dog 4-71) are graphically illustrated in chart 1. It is quite clear that the total globulin curve approaches nearest to that of the whole exudate. A diagrammatic summary of the effects of the various fractions of exudate on the leukocyte level is shown in chart 2. It is interesting to note that the albumin fraction per se induces an actual lowering in the circulating white cell count as compared with the normal range of fluctuation. On the other hand, the effect of the total globulin fraction is even more pronounced than that of the exudate (columns 2 and 3, chart 2). It is therefore conceivable that this may in part be ascribed to the relative absence of albumin in the fraction obtained by salting out with ammonium sulfate at half saturation. It is perhaps worth while to point out here that the volume of untreated exudate injected was comparable in quantity to that employed in the fractionation and subsequent testing of the material.

Longsworth, Shedlovsky and MacInnes¹⁰ recently called attention to the increase in alpha globulin as shown by electrophoretic measurements and to the consequent high value of the resulting proportion of alpha globulin to albumin in the blood serum of patients afflicted with various inflammatory processes. The alpha globulin is presumably in

10. Longsworth, L. G.; Shedlovsky, T., and MacInnes, D. A.: *J. Exper. Med.* 70:399, 1939.

the pseudoglobulin fraction. It is therefore conceivable that the findings of these investigators were due to an excess of globulin containing the leukocytosis-promoting factor that had in turn penetrated from the site of inflammation into the circulating blood stream. It is to be recalled at this point that the factor, whether purified or in the untreated exudate, seems to induce a discharge of immature granulocytes from the bone marrow into the circulation.^{3b} This suggests an increase in the level of the leukocytosis-promoting factor in the circulation of animals with acute inflammatory lesions accompanied by leukocytosis. It is also possible that the leukocytosis-promoting factor may prove to have clinical application in some infectious conditions. Studies are now in progress in an endeavor to study these various questions and also in an attempt to purify further the leukocytosis-promoting factor of exudates.

SUMMARY

There exists in inflammatory exudates a factor capable of inducing marked increase in the number of circulating leukocytes. The presence of this leukocytosis-promoting factor is readily demonstrated by noting the effect of injected exudate on the leukocyte level of a normal animal. Earlier studies have shown that various other, unrelated materials, including blood serum, broth, turpentine, bacteria, nucleic acid derivatives, histamine and leukotaxine, are wholly ineffective in reproducing in dogs precisely the same pattern of response as exudates produce.^{3b} The leukocytosis-promoting factor bears no evident relationship to leukotaxine, the nitrogenous substance responsible for the increase in capillary permeability and leukocytic migration.

The leukocytosis-promoting factor is thermolabile and essentially nondiffusible. Observations on protein fractionation of exudates either after dialysis or after "salting out" of the material at various concentrations of ammonium sulfate indicate either that the leukocytosis-promoting factor is a globulin or that at least it is closely associated with that protein fraction. The available data warrant the belief that the factor seems linked primarily with the pseudoglobulin fraction of inflammatory exudate.

Studies are in progress in an effort to purify further this active globulin-like substance which per se offers an explanation for the mechanism of leukocytosis accompanying inflammation.

TOXOPLASMA INFECTION IN MAN

HENRY PINKERTON, M.D.

AND

DAVID WEINMAN, M.D.

ST. LOUIS

A fatal generalized infection with a protozoan parasite was encountered in a series of postmortem examinations made in Lima, Peru. Although this infection was not transmitted to animals, a careful comparison of the morphologic and histologic features with those of various protozoan infections in man and experimental animals has led us to the conclusion that the organism in question is *Toxoplasma*. The identification of this organism, and its differentiation from other protozoa with which it might be confused, will be discussed in this paper.

HISTORICAL REVIEW

Toxoplasma infection was probably first seen in Java sparrows (*Padda oryzivora*), by Laveran,¹ in 1900. Later this organism was described by Splendore² in rabbits, and by Nicolle and Manceaux³ in the north African rodent *Ctenodactylus gondii*.

An early suggestion that organisms of the genus *Toxoplasma* might be concerned in human disease was contained in a case report published by Castellani⁴ in 1913 and 1914. This case concerned a Singhalese, aged 14, who suffered from splenomegaly, prolonged fever, anemia and leukopenia. Death occurred about four weeks after hospitalization. Structures believed to be *Toxoplasma* were seen in two blood smears made during life and in impression smears of the spleen made after death. No histologic lesions in the spleen or other organs were described. The name *Toxoplasma pyrogenes* was proposed for these bodies.

Wenyon⁵ refused to accept these structures as protozoa, believing that the larger forms described were degenerating leukocytes and the smaller forms yeasts. He stated: "It is the writer's opinion that the

From the Department of Pathology, St. Louis University School of Medicine, and the Department of Comparative Pathology and Tropical Medicine, Harvard Schools of Medicine and Public Health.

1. Laveran, A.: *Compt. rend. Soc. de biol.* **52**:19, 1900.

2. Splendore, A.: *Rev. Soc. scient. de São Paulo* **3**:109, 1908.

3. Nicolle, C., and Manceaux, L.: *Arch. Inst. Pasteur de Tunis*, 1909, p. 97.

4. Castellani, A.: *J. Ceylon Br. Brit. M. A.* **10**:20, 1913; *J. Trop. Med. & Hyg.* **17**:113, 1914; *J. Ceylon Br. Brit. M. A.* **11**:45, 1914.

5. Wenyon, C. M.: *Trop. Dis. Bull.* **20**:527, 1923.

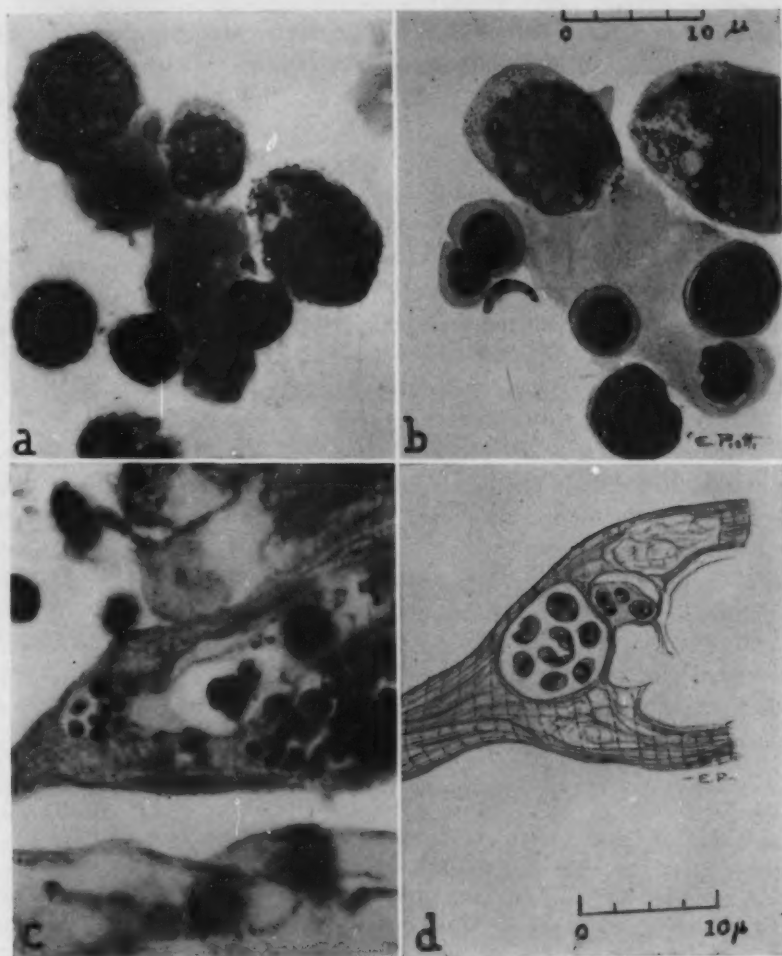


Fig. 1.—(a) A single crescent-shaped form of *Toxoplasma* in a Giemsa-stained impression film of the bone marrow in the case reported; $\times 1,200$. (b) Drawing of the same; $\times 1,500$. (c) *Toxoplasma* in a cardiac muscle fiber from the edge of a focal area of necrosis; eosin-methylene blue stain after fixation of tissue in Zenker's fluid; $\times 1,200$. The two organisms in the center of the smaller group are joined together at one end, probably representing recent incomplete division. (d) Drawing of a portion of the field illustrated in c, showing the internal structure of the organisms; each has an irregular basophilic nucleus and eosinophilic cytoplasm.

organism called *Toxoplasma pyrogenes* is neither a *Toxoplasma* nor a protozoön, and that the name is a *nomen nudum*, as it is given to certain unidentifiable vegetable cells."

Certain illustrations which accompany Castellani's papers show structures which closely resemble some forms of *Toxoplasma*. The structures were not reported as having occurred in aggregates, however, and a description of histologic changes in association with them is lacking. Thus, from the evidence available, it appears that their nature must remain in doubt. In any case there seems to be no justification for giving a new specific name to structures so incompletely identified.

Fedorovitch⁶ studied a child with anemia, splenomegaly and slight hepatomegaly and found extracellular structures in the blood which he considered to be analogous to those described by Castellani and very similar to *Toxoplasma*. They were not found in material obtained by splenic puncture. From the scanty description and the illustrations, it seems possible that these may have been *Toxoplasma*. Wenyon⁵ stated: "The colored figures given by Fedorovitch leave no doubt that the bodies were vegetable organisms like large cocci and yeast."

Chalmers and Kamar⁷ reported observations on a soldier dying of an unidentified disease characterized by fever, splenomegaly, anemia and bleeding from the gums. In films of spleen, bodies were seen which were stated to be "comparable" with "*T. pyrogenes*." The illustrations show bodies which are difficult to interpret. Wenyon⁵ stated that "the structures seen in this case were altered *Leishmania*."

More recently a predominantly cerebral infection with a *Toxoplasma*-like organism has been described in 5 infants, and in each case the parasites were demonstrated in the lesions in such a way as to leave no doubt concerning their etiologic significance.

1. Janků's case⁸ was that of an infant about 1 year of age. Left microphthalmia was noted three days after birth, and apparent blindness from the age of 3 months, followed by progressive hydrocephalus, spastic contractures of the limbs and irregular generalized convulsions with nystagmoid movements of the eyes. The spleen was not palpable, and the temperature was normal. On ophthalmoscopic examination there was seen in each eye a white area in the vicinity of the macula. The hydrocephalus increased, and the child died. The brain was available only for gross examination. The aqueduct of Sylvius was found to be completely obliterated. Sections of the eyes showed extensive lesions of the retinas. The lesions of the right eye showed collections of parasites. The individual parasite was an oval body with eosinophilic cytoplasm and a basophilic nucleus. Janků did not classify

6. Fedorovitch, A. I.: *Ann. Inst. Pasteur* **30**:249, 1916.

7. Chalmers, A. J., and Kamar, A.: *J. Trop. Med. & Hyg.* **23**:45, 1920.

8. The details of this case are taken from an English summary (Wolf, A., and Cowen, D.: *Bull. Neurol. Inst. New York* **6**:306, 1937) of J. Janků's original papers in Czech (*Časop. lék. česk.* **62**:1021, 1054, 1081, 1111 and 1138, 1923).

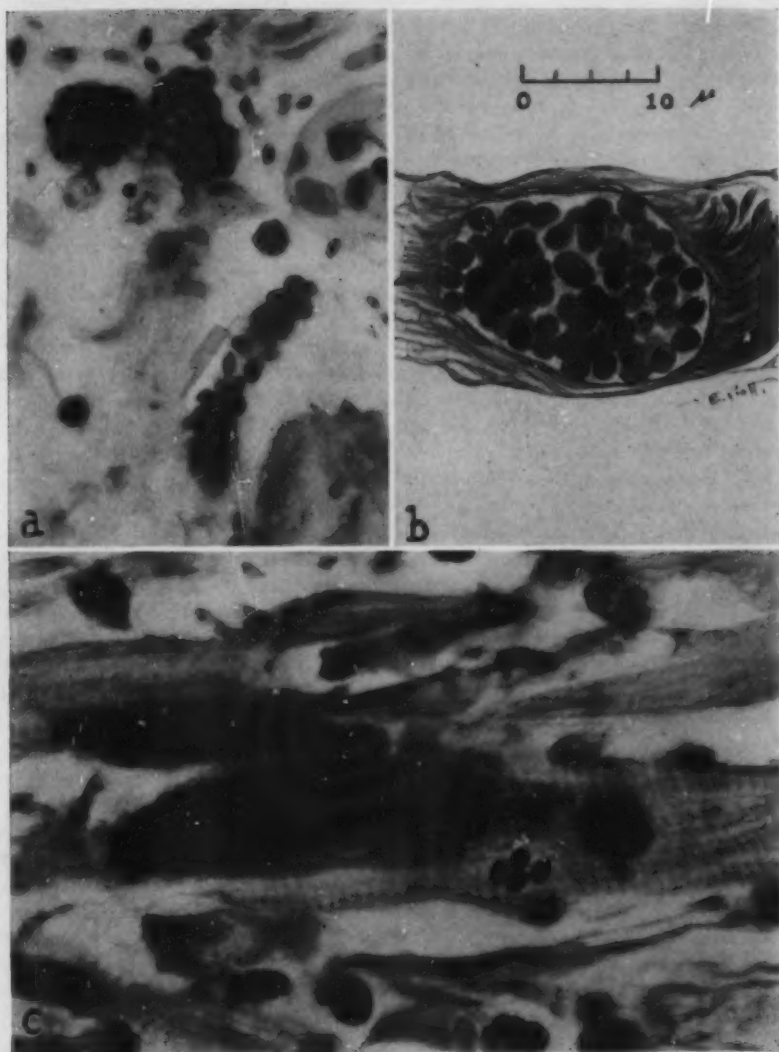


Fig. 2.—Collections of the parasites in myocardial lesions in the case reported; Reguad's fluid followed by Giemsa stain. (a) Photomicrograph showing three groups of organisms, probably set free by degenerated muscle fibers; $\times 1,200$. (b) Drawing of a spherical group of organisms in a muscle fiber; $\times 1,500$. (c) Photomicrograph of an elongated group of organisms in a muscle fiber cut longitudinally. Note the group of four organisms below the right hand edge of the large group. These appear larger than those in the large group; $\times 1,200$.

this parasite generically, but Wolf, Cowen and Paige⁹ considered it to be *Toxoplasma*, and the present authors concur in this view.

2. Torres¹⁰ reported the postmortem observations on an infant who showed generalized muscular contractures at birth and died with convulsions two days later. Both hemispheres contained disseminated yellow nodules of pinhead or smaller size. The leptomeninges were thin and transparent. Microscopically, focal necroses containing compact masses of parasites were found in the brain, in the skeletal and cardiac muscle fibers and in the fatty subcutaneous tissue. These masses were intracellular and were found within the lesions. Large aggregates, such as those occurring in muscle fibers, measured as much as 41 by 10 microns; the average size, however, was in the neighborhood of 12 by 7 microns. Each aggregate was composed of "ovoid corpuscles," averaging 3.5 by 1.5 microns, the largest forms measuring 6 by 1.5 microns. The individual "body" contained a round nucleus, measuring 1 micron in diameter, with chromatin localized at the periphery. The inflammatory cells were chiefly mononuclears, although eosinophils were sometimes abundant. No extracellular forms were observed. The parasite was at first^{10a} considered to be either *Encephalitozoon* or *Toxoplasma*. Later^{10b} Torres chose to classify it "provisionally" as *Encephalitozoon*. The reasons given for this classification were (1) that it was smaller than *Toxoplasma cuniculi*, found in rabbits, (2) that it had a syncytial phase, while *Toxoplasma* was supposed to multiply only by binary division and (3) that it occurred exclusively within cells, whereas *Toxoplasma* was believed to have many extracellular forms.

The discrepancy in size stressed by Torres was apparently based on a comparison of his organism in sections with measurements given for *T. cuniculi* in films. Under comparable conditions this discrepancy would probably not have been found. Multiple division of a schizogenous type has been described for *Toxoplasma* by Splendore¹¹ and by Chatton and Blanc¹² and was considered by França¹³ a distinguishing feature of the *Toxoplasma* family. *Encephalitozoon* and *Toxoplasma* have both been described as occurring intracellularly and extracellularly. In view of these facts, as well as the morphologic features of the organism described by Torres, it seems probable that this organism was in reality *Toxoplasma*.

3. In 1937 Wolf and Cowen⁸ studied an infant who presented an enlarged cranium, fever, a rapid pulse, hyperactive reflexes with a bilateral Babinski sign, and convulsive seizures. The cerebrospinal fluid was xanthochromic and showed a 4+ globulin reaction; the cell count ranged between 200 and 1,500, the majority being mononuclear cells; at times red blood cells were present. The left ventricular pressure was slightly increased. No growth was obtained from cultures of ventricular fluid, but the precipitate obtained from this fluid contained parasites. Blood cultures gave no growth. The blood leukocyte count was 7,800, of which 52 per cent were lymphocytes. The urine contained acetone and diacetic acid in large quantities but no sugar. Ophthalmologic examination showed bilateral abnormalities of the retina, consisting of elevated, yellowish white areas.

Death occurred at 4 weeks of age. The aqueduct of Sylvius was almost completely occluded. Pathologic changes were observed in the brain, spinal cord,

9. Wolf, A.; Cowen, D., and Paige, B.: *Science* **89**:226, 1939.

10. Torres, C. M.: *Compt. rend. Soc. de biol.* (a) **97**:1778, (b) 1787 and (c) 1797, 1927.

11. Splendore, A.: *Bull. Soc. path. exot.* **2**:462, 1909.

12. Chatton, E., and Blanc, G.: *Arch. Inst. Pasteur de Tunis* **10**:1, 1917.

13. França, C.: *J. sc. math., fis. e nat.* **1**:26 and 221, 1917.

retinas and choroids. The microscopic lesions, which were all similar, consisted of granulomas and areas of necrosis and infiltration. These lesions were observed to contain micro-organisms.

The parasites occurred intracellularly and extracellularly, singly and in aggregates. In fixed tissue sections the majority of the single organisms measured between 2 and 3 microns in length and 1.5 to 2 microns in width. In shape the

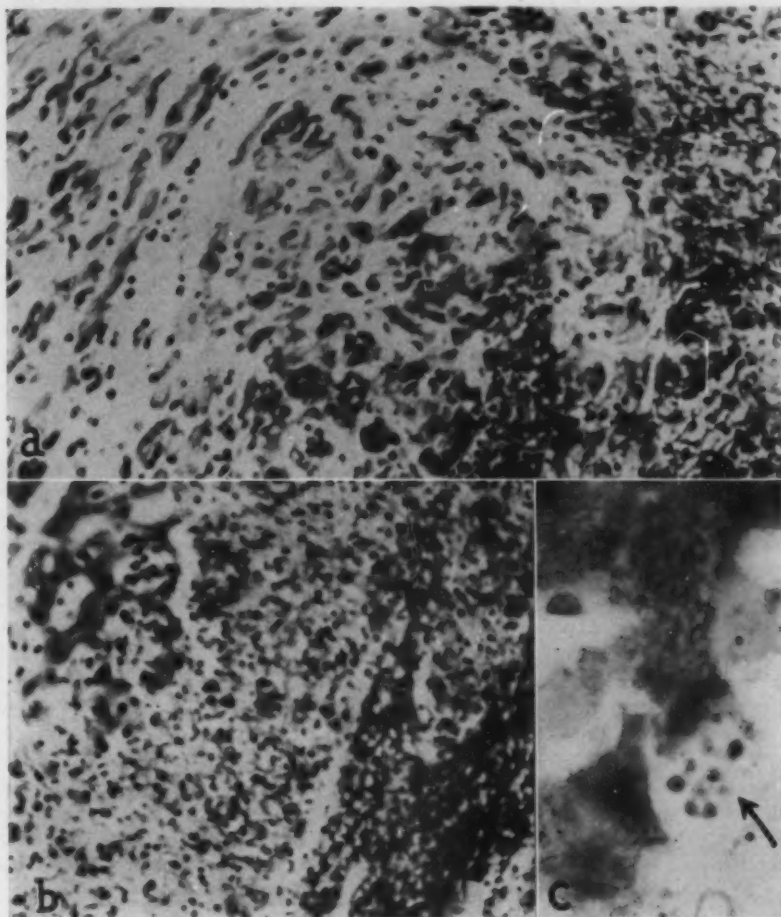


Fig. 3.—(a) Section through the edge of an area of myocardial necrosis, showing essentially normal heart muscle fibers at the left and fibrinoid degeneration and inflammatory cell infiltration at the right (case reported); eosin-methylene blue stain; $\times 300$. (b) Section through the edge of a similar necrotic lesion in the liver; eosin-methylene blue stain; $\times 300$. (c) A small group of organisms in a Kupffer cell from the lesion shown in b; $\times 1,200$.

majority were ovoid or oval, but some were piriform. The chromatin mass was large, eccentric, often polar, and stained more deeply at the periphery than in the center. The cytoplasm appeared pink or red after eosin-methylene blue staining;

it was not represented as containing vacuoles. The collections varied from 6.8 by 6 microns to 15.5 by 17 microns in dimension and were composed of numbers of single organisms.

The parasite was at first classified as a new species of *Encephalitozoon*, but the authors later considered it to be a species of *Toxoplasma*,⁹ for which the specific name *hominis* was proposed. It appears that this organism is correctly classified as a *Toxoplasma*, but at present there is not sufficient evidence that it constitutes a new species, since criteria which would permit differentiating it from the species of *Toxoplasma* found in animals have not been furnished (see later comment on this).

4. Richter¹⁴ presented the pathologic observations on an infant dying from "meningo-encephalomyelitis neonatorum" of infectious nature but of undetermined cause. Wolf and Cowen¹⁵ later were able to identify parasites within the lesions. Fever had been noted for five days and convulsions for one day before hospitalization. The infant died on the day of admission, after having exhibited opisthotonos, convulsive twitchings and clonic spasms of the arms and legs. The temperature was 101.6 F. The spinal fluid was xanthochromic, contained 30 white and 880 red blood cells per cubic millimeter, and the protein content was 1,260 mg. in 100 cc. The report of the examination of the blood was: hemoglobin 60 per cent; red cells 3,580,000 and white cells 54,000 per cubic millimeter; neutrophils 23 per cent, small mononuclears 50 per cent, large mononuclears 10 per cent, eosinophils 3 per cent, basophils 2 per cent, normoblasts 2 per cent and unclassified leukocytes 3 per cent.

Death took place at the age of 7 weeks. Aside from terminal bronchopneumonia, significant changes were observed only in the nervous system. They are summarized as disseminated miliary granulomas of the central nervous system similar to those in previously reported cases. A focal leptomeningeal reaction was also present. Within these lesions micro-organisms occurred which the authors described as "morphologically identical" with those seen in their previous case.⁸

5. Wolf, Cowen and Paige⁹ reported a third case. A child became ill at 3 days of age, presenting convulsive seizures and symptoms of involvement of the spinal cord. Ophthalmoscopically, irregular, reddish brown areas were observed in each retina. Death occurred at the age of 31 days. Autopsy material was limited to the brain and eye. The brain revealed widespread encephalomyelitis, characterized by disseminated miliary granulomas and focal areas of inflammation and necrosis. The right eye showed localized chorioretinitis. A protozoon "morphologically identical with *Toxoplasma* was present in all the lesions." Animals were inoculated with fresh brain and spinal cord tissue. Rabbits, infant mice, newly hatched chicks and guinea pigs died following intracerebral inoculation of the infected human nerve tissue. The infected animals showed both lesions and parasites resembling those in the patient. Rats and a monkey (*Macacus rhesus*) which were inoculated intracerebrally gave no subsequent evidence of infection. Certain rabbits and mice which did not die from the original infection were reinoculated intracerebrally and survived, thus showing acquired immunity. They were finally inoculated intracerebrally and intraperitoneally with a strain of *Toxoplasma* obtained from guinea pigs by Sabin and Olitsky,¹⁶ and again they survived. Other rabbits immunized against the guinea pig strain withstood subsequent inoculation with the human strain, whereas nonimmunized controls succumbed.

14. Richter, R.: Arch. Neurol. & Psychiat. **36**:1085, 1936.

15. Wolf, A., and Cowen, D.: Bull. Neurol. Inst. New York **7**:266, 1938.

16. Sabin, A. B., and Olitsky, P. K.: Science **85**:336, 1937.

Sabin¹⁷ obtained mice infected with this human strain and arrived at results of the same order. He stated that "*Toxoplasma* of animal and human origin have been shown to be identical biologically . . . and immunologically."

Reference to Bland's strain of *Toxoplasma*¹⁸ may be encountered in the literature, accompanied by indications of its isolation from man. Blood removed from

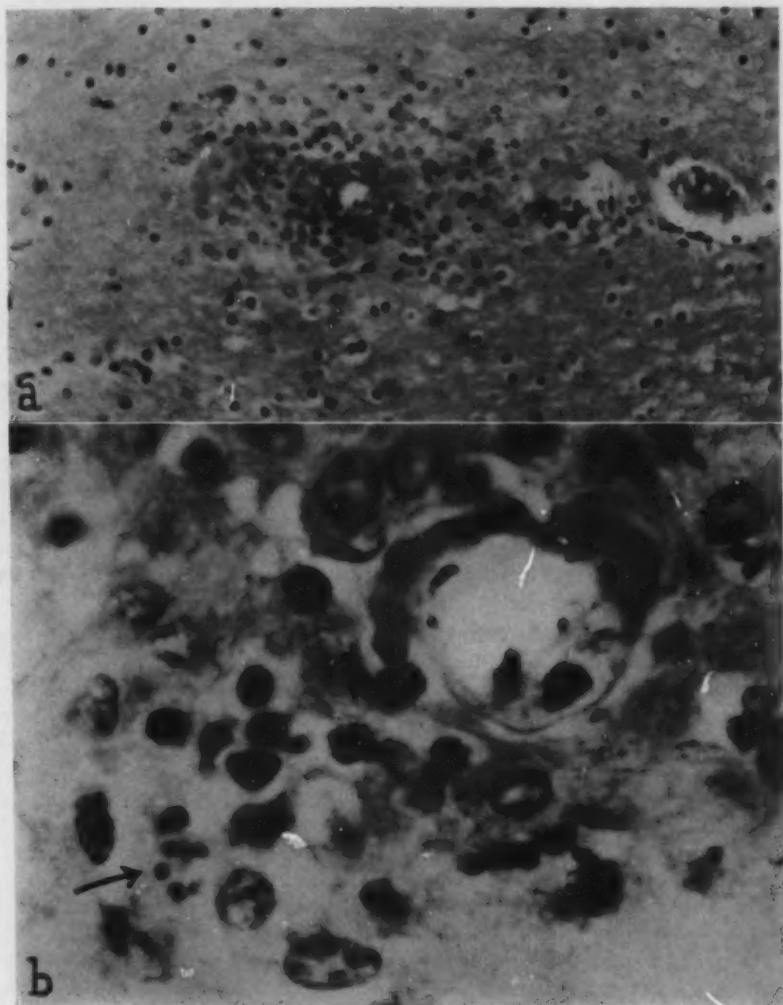


Fig. 4.—(a) Focal perivascular lesion in the internal capsule of the brain (case reported); eosin-methylene blue stain; $\times 250$. (b) Higher magnification of the central portion of the field shown in a. The arrow points to a small collection of parasites apparently lying free in the brain substance; $\times 1,200$.

17. Sabin, A. B.: Proc. Soc. Exper. Biol. & Med. **41**:75, 1939.

18. Bland, J. O. W.: Lancet **2**:521, 1930; Brit. J. Exper. Path. **12**:311, 1931.

several patients suffering from infectious mononucleosis was inoculated into rabbits. One of the inoculated rabbits became ill, and blood from the affected rabbit permitted the disease to be carried on by serial passages. Later it was found that the rabbit disease was caused by *Toxoplasma*. It was stated that this strain of *Toxoplasma* was immunologically identical with *Toxoplasma cuniculi*. It was also stated that the bodies of *Toxoplasma* were found in a control rabbit. It was nowhere stated that they were observed in the original human blood. Nonetheless, the author concluded: "... the evidence suggests that human glandular fever may be caused by the protozoa described, but this requires confirmation." It seems at least equally plausible to suppose that these bodies were of rabbit and not of human origin.¹⁹

These 5 cases in which parasites were demonstrated post mortem in sections of human tissue possess certain clinical features in common; these are summarized in table 1, the 2 cases to be described in this paper being added for completeness.

REPORT OF A CASE

A 22 year old Peruvian entered the service of Dr. F. Salazar Alarco (who gave us the following clinical history) in the Hospital Dos de Mayo, Lima, Peru, March 24, 1937, complaining of weakness, pallor, and fever of one week's duration. March 26 his temperature was 39.4 C. (102.9 F.), and blood films showed a moderately heavy infection of the erythrocytes with *Bartonella bacilliformis*. The erythrocyte count was 1,060,000 and the white cell count 10,000. The differential count was given as: neutrophils (including 3 per cent myelocytes) 88 per cent, monocytes 6 per cent and lymphocytes 6 per cent. March 26 bartonellas had almost disappeared from the blood and were found only with difficulty. In spite of this fact, usually considered of good prognostic import, the patient failed rapidly and died at 8:30 a. m. March 29.

Necropsy.—The body was well developed and fairly well nourished, but the skin and mucous membranes showed marked pallor. The inguinal and axillary lymph nodes were definitely enlarged. Small colorless subcutaneous papular nodules, averaging about 3 mm. in diameter and 1 mm. in height, were sparsely distributed over the chest, back and upper parts of the legs. About 25 cc. of clear yellowish fluid was present in each pleural cavity.

The heart was dilated but not hypertrophied. A few petechial hemorrhages were noted on the surface of the right auricle. On the surface of the right ventricle were several grayish areas of necrosis, the largest of which was 8 by 4 mm. On section, many similar areas of necrosis, averaging 2 to 3 mm. in diameter, were seen. Several of these were yellowish and they had somewhat the appearance of recent infarcts. A few similar lesions were seen in the left ventricle.

The pleural surfaces of both lungs were studded with petechial hemorrhages. The lungs showed congestion and edema but no other definite gross changes. The bronchial lymph nodes were moderately enlarged.

The spleen was markedly enlarged, measuring 8 by 3½ by 2¼ inches (20 by 8.75 by 5.5 cm.). Both externally and on section it showed many opaque, yellowish

19. In regard to the suggested relationship between human *Toxoplasma* infection and infectious mononucleosis, it may be noted that Sabin¹⁷ was unable to demonstrate neutralizing antibodies against *Toxoplasma* in the blood of a number of patients recovering from infectious mononucleosis.

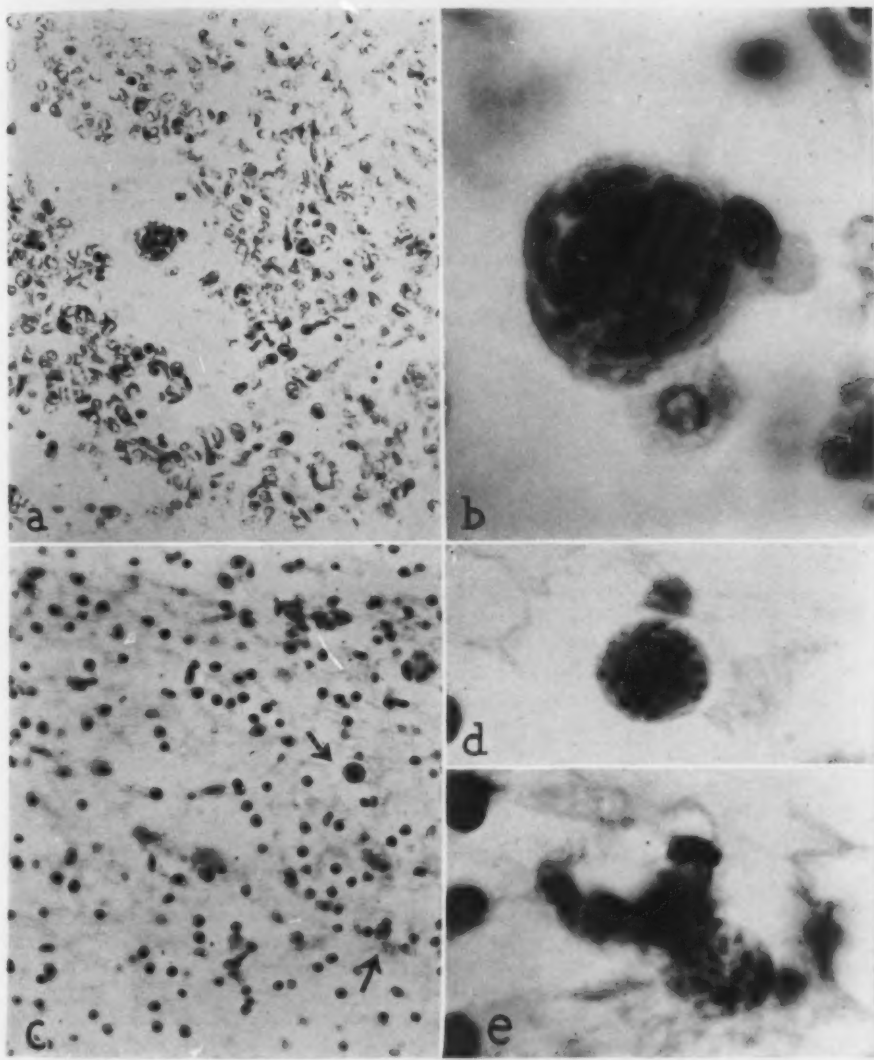


Fig. 5 (case reported by Hertig in 1934 and restudied by present authors).— (a) Lung showing an intracellular collection of parasites, probably within a macrophage which lies apparently free in an alveolus; hematoxylin and eosin stain; $\times 320$. (b) Higher magnification of the parasites shown in a; $\times 1,500$. (c) Cerebral cortex showing inflammatory cell infiltration and collections of parasites indicated by arrows; hematoxylin and eosin stain; $\times 320$. (d) and (e) Higher magnification of parasites shown in c.

white nodules, ranging up to 1.5 cm. in diameter, and similar to the lesions noted in the myocardium. The smaller lesions somewhat resembled miliary tubercles, while some of the larger ones were wedge shaped and resembled infarcts.

TABLE 1.—Summary of Reported Cases of Undoubted *Toxoplasma* Infection

Case Reported	Age at Death	Fever	Spleno- megaly	Hydro- cephalus	Anemia	White Blood Cells	Con- vul- sions	Retinal Lesions Ob- served During Life	Organs Containing Lesions and Parasites	Other Observa- tions
Jankú, 1923 ^a	About 1 year ^b	0	0	+	?	?	+	+	Eye; other organs not examined	Microph- thalmia; blind- ness
Torres, 1927 ¹⁰	2 days	?	?	?	?	?	+	?	Central ner- vous system; myocardium; skeletal mus- cle; subcutis; none in spleen, liver and kidney
Wolf and Cowen, 1937 ^a	4 weeks	+	0	+	0	7,800 with relative lymphocy- tosis (52 per cent lympho- cytes)	+	+	Central ner- vous system; eye; none in heart, liver, spleen and skin
Richter, 1936, ¹⁴ and Wolf and Cowen, 1938 ¹⁴	7 weeks	+	?	?	†+ 54,000, with 50 per cent lympho- cytes and 23 per cent neutro- phils	+	+	?	Central ner- vous system, none in other organs	Terminal broncho- pneu- monia
Wolf, Cowen and Paige, 1939 ^a	31 days	?	?	?	?	?	+	+	Central ner- vous system; other organs not exam- ined
Hertig, 1934 ³⁰	25 days	+	0	0	?	18,500	0	..	Central ner- vous system; heart; lungs; adrenal	Multiple purulent bacterial foci; terminal broncho- pneu- monia
Pinkerton and Wein- man, 1940	22 years	+	+	0	+	10,000, with relative lympho- penia	0	?	Central ner- vous system; myocardium; liver; spleen; skin; lymph nodes; bone marrow, adre- nals, kidney	Concomi- tant bar- tonella infection

^a It is not clear whether the patient died at 11 or 16 months of age.

† Slight.

+ The condition was reported to be present.

? The condition was not mentioned.

0 The condition was reported not to have been found or observed.

The liver was studded, both externally and on section, with similar lesions, 1 to 3 mm. in diameter, again resembling miliary tubercles, but more irregular in shape.

The gastrointestinal tract was normal except for petechial hemorrhages in the mucosa of the stomach. The mesenteric, retroperitoneal and inguinal lymph nodes

were enlarged to two or three times normal size, and on section were soft, succulent and mottled with areas of hemorrhage and irregular, opaque yellowish areas of necrosis.

The kidneys, pancreas and adrenals were grossly without change.

The brain was normal except for congestion and some flattening of the convolutions.

Microscopic Observations.—(a) Heart: The necroses described grossly were seen microscopically to be circumscribed foci of coagulation necrosis, with partial or complete disappearance of the outlines of muscle fibers and considerable deposition of fibrin. Thrombi composed of leukocytes and fibrin were numerous in the capillaries and precapillaries, particularly in the peripheral portions of the lesions. A heavy cellular infiltration, predominantly neutrophils and eosinophils, was seen in the central portions of these lesions; mononuclear cells were more numerous peripherally. A protozoon (to be described later) was present in great numbers, frequently within the cytoplasm of cardiac muscle fibers, at times in large collections easily seen on examination with the lower power objective. These intracellular collections were frequently seen at the edges of the lesions, where they often occurred in apparently normal muscle fibers, but never in the normal-appearing regions of the myocardium at a distance from the lesions. These parasites were also seen, apparently free, in the central portions of the lesions, singly and in small groups. In some instances small capillaries seemed to be occluded with organisms. Large portions of the myocardium were apparently normal, but there were also, in addition to the necrotic foci described, regions in which the picture was that of diffuse myocarditis. In these areas lymphocytes, macrophages and eosinophils were present between the muscle fibers and in the interstitial connective tissue. Not infrequently, focal collections of large mononuclear cells, some of which were multinucleated, were seen around blood vessels, giving a picture somewhat suggestive but not entirely typical of Aschoff bodies. Organisms were only rarely seen in these foci of milder injury.

(b) Lungs: Many alveoli were filled with serous exudate, and a few foci of neutrophilic infiltration were seen. Occasional collections of protozoa were present in mesenchymal cells of the alveolar walls, not always in association with groups of neutrophils.

(c) Spleen, Lymph Nodes, Adrenals, Kidneys, Liver: The lesions in these organs were similar in nature, but those in the adrenals and kidneys were smaller and less numerous than those in the other organs. In order to avoid repetition, only the lesions in the liver will be described. The lesions in this organ were roughly spherical, from about 0.2 to 3 mm. in diameter, and sharply circumscribed. Their distribution seemed unrelated to the anatomic units of the organ. The smaller lesions as seen in sections consisted of 15 to 20 liver cells with eosinophilic cytoplasm and pyknotic nuclei, surrounded by inflammatory cells, chiefly neutrophils. Each of the larger lesions showed a necrotic, almost caseous-appearing central portion with many degenerating neutrophils. Parasites were abundant in Kupffer cells, occasionally present in liver cord cells at the edges of the lesions and scattered extracellularly in the necrotic central portions of the lesions.

(d) Brain: Rare focal collections of neutrophils and small round cells, usually in the walls of small capillaries, were found, and in one of these lesions a group of parasites was seen, apparently located extracellularly.

(e) Skin: Sections of the subcutaneous nodules showed that the parasites occurred in the deeper layers of the corium and the subcutaneous fatty tissue. The lesions, like those elsewhere, were essentially foci of tissue necrosis and

fibrin deposition, with a heavy infiltration of neutrophils and other inflammatory cells. The protozoon was present, in large numbers, in macrophages and fat cells; also as extracellular collections and small groups that were apparently in the lumens of small capillaries. Endothelial proliferation and fibrin thrombi within small blood vessels were also conspicuous features of these lesions.

(f) *Striated Muscle*: Several sections of striated muscle from the thigh showed no lesions or parasites.

MORPHOLOGIC CHARACTER OF THE PARASITE

The most satisfactory methods of demonstrating the parasite in sections were the use of Giemsa's stain after Regaud's fluid and the use of eosin-methylene blue after Zenker's fixative. With either method quite similar results were obtained, and the following description is based on a study of sections prepared by these methods.

The parasites were found as agglomerations (pseudocysts or collections) or as isolated organisms. The agglomerations varied in size according to the number of individual bodies which composed them, and their size as well as their shape appeared to depend in part on the nature of the tissue in which they developed. The largest masses occurred in the cardiac muscle fibers. One such mass was spindle shaped, measuring 51 by 10 microns. Another large mass was round, measuring 32 by 34 microns; it occurred at the point of bifurcation of a heart muscle fiber. These larger collections in the myocardium were found in only slightly altered muscle fibers, where the cross striations were usually still visible, and they were situated within the boundaries of one, or if growth extended through a region of bifurcation, two muscle fibers. In the necrotic portions of the heart, and in the other organs, the masses were more frequently rounded. A small mass (composed of eight organisms) measured 5.1 by 5.1 microns. A mass composed of fourteen to sixteen elements, a common number, measured 10.2 by 7.6 microns.

The intracytoplasmic agglomerations were often surrounded by a narrow clear halo. Frequently they were juxtanuclear, and at times they were in contact with the nuclear membrane, which they indented. Certain masses were entirely surrounded by a fine smooth line, which appeared to be derived from the adjacent host tissue; internal septums were not observed. Some of the agglomerations, particularly the larger ones in the heart muscle, contained a few dark disks interspersed among the parasites. These disks, measuring about 4 microns in diameter and staining like chromatin, resembled the altered nuclear material of adjacent host cells.

Individual organisms, seen lengthwise, appeared as crescentic, ovaliform, elliptic or planoconvex bodies, with the nuclei fairly well differentiated from the cytoplasm. Their longest diameter was usually between 3.4 and 4.3 microns, and their shortest, between 1.3 and 1.7 microns.

The largest specimen seen measured 4.3 by 2.2 microns. The dimensions varied somewhat, and specimens lying in loose tissue or loosely packed in vacuoles were often larger than those closely packed in dense masses. In transverse section the organisms were circular.

The cytoplasm was pink after eosin-methylene blue staining; with the Giemsa stain it was pink or pale blue, according to the degree of differentiation. No specimen contained pigment. The shape of the extremities was quite variable; seen lengthwise both extremities usually tapered, but one often appeared more rounded than the other. Frequently both ends were equally rounded, and occasionally the tapering did not occur.

Nuclei were present in all specimens, but occasionally in transverse sections no cytoplasm was visible. The nucleus occupied nearly the entire width of the organism, and the rounded forms measured 1.3 to 1.7 microns in diameter. In the fixed material the nuclei were often irregular in shape, usually nearer to one end and almost always on the longitudinal midline. They stained blue, and in their interior one or more chromatic granules could be seen in certain well stained specimens, while in some organisms the nuclei appeared distinctly stippled.

Multiplication of the parasite apparently takes place by binary division. Binucleated forms, believed to have been fixed in a state of division, appeared more rounded than the other organisms. In such forms the more nearly plane surfaces faced one another, the nuclei of the daughter cells being at the same end.

Parasites were not encountered after long search in blood films made during life and at the postmortem examination. An impression film of the femoral bone marrow was available. In this film, which was air dried and stained by the Giemsa method, a single parasite was detected. This organism was crescentic, measuring 5.1 by 1.3 microns. The nucleus stained red-violet, occupied the entire width of the cytoplasm and was stellate. A single red-staining granule was found within the cytoplasm near one end.

COMMENT

Generic Identification of the Parasite.—The organism described in this report bears a superficial resemblance to *Leishmania* and to *Trypanosoma cruzi* (aflagellate forms), but each of these organisms contains in its cytoplasm, in addition to the nucleus, a prominent rod-shaped kinetoplast. The constant occurrence of this structure distinguishes these organisms sharply from the protozoon described here.

Sarcocystis has been reported in man. It is found in striated muscle and connective tissue, where it produces cysts, which at their full development are easily seen by the unaided eye. These cysts may produce slight degenerative changes in the invaded tissue, but there is no massive

necrosis or inflammatory infiltration. The cysts are limited by a definite membrane, at times several microns thick, and frequently are subdivided by internal septums. The individual spores seen in section are falciform and uninucleate. The differences between *Sarcocystis* and *Toxoplasma* are shown in table 2.

The principal difficulty in the differential diagnosis occurs with the parasites classified as *Encephalitozoon*. These parasites were seen, described and illustrated by Wright and Craighead.²⁰ They were studied in detail and named by Levaditi, Nicolau and Schoen.²¹

The type species of this genus, *Encephalitozoon cuniculi*,²² infects a variety of hosts, i. e., mice, rats and dogs, and produces nodules, which

TABLE 2.—Comparison of *Toxoplasma* and *Sarcocystis*

	<i>Toxoplasma</i>	<i>Sarcocystis</i>
Type of development	Collections contain parasites either all at same stage or some young and some adult, intermingled	Prolonged period of multiplication within cyst, producing mature spores in central portion and immature spores at peripheral or terminal portion
Size of largest aggregates	Microscopic, rarely above 50 to 60 microns long	Macroscopic, several millimeters or more in diameter
Organization of aggregates	Limiting membrane, never thick and striated; internal septums absent	Limiting membrane, sometimes thick and striated; internal septums present
Tissue or organ invaded (after initial dissemination)	Little restricted; found in various tissues—brain, skin, heart, liver, spleen, etc.	Quite restricted; striated muscle and connective tissue
Cellular position	Intracellular and extracellular	Intracellular
Pathogenicity in cases of extensive invasion	Marked, usually acutely fatal for host	Usually slight, may produce cachectic state
Lesions of the host in cases of extensive invasion	Usually extremely marked; foci of necrosis and leukocytic infiltration	Usually absent; sometimes muscular degeneration

may be necrotic and accompanied by infiltration, in various organs of the infected animals. It is described as a small oval organism; the measurements are given as 2.5 microns by 0.5 to 1 micron, the maximum length and width being not over 4 by 1.5 microns.²⁰ The individuals are found isolated or grouped in "cysts" composed of numerous elements and measuring up to 20 by 30 microns.²¹

The resemblance of this organism to *Toxoplasma* is striking. Through the assistance of Dr. E. E. Tyzzer, we have had an opportunity to study an *Encephalitozoon* found as a spontaneous parasite in sections of a kidney of a mouse. Dr. Sabin has furnished us with sections showing *Toxoplasma* infection in laboratory animals. From a study of this material and from published descriptions, the distinguishing features

20. Wright, J. H., and Craighead, E. M.: *J. Exper. Med.* **36**:135, 1922.

21. Levaditi, C.; Nicolau, S., and Schoen, R.: *Ann. Inst. Pasteur* **38**:651, 1924.

22. Nicolau, S., and Balmus, G.: *Compt. rend. Soc. de biol.* **113**:1002, 1933.

between Encephalitozoon and Toxoplasma appear to be the following: 1. Encephalitozoon is smaller and more uniformly elliptic; Toxoplasma is larger and more varied in shape, with tapering extremities, of which at least one is often pointed. 2. Encephalitozoon may often show no nucleus-like structure, while a nucleus is always present in Toxoplasma. 3. Encephalitozoon is surrounded at its periphery by a fine continuous line, giving the appearance of a limiting membrane. 4. Encephalitozoon shows clear regions of varying extent, and often the entire area within the "limiting membrane" appears empty, while the cytoplasm of Toxoplasma is always stained and most often not vacuolated.²³

The application of these criteria indicates quite clearly that the parasite reported in this paper has the characteristics of Toxoplasma rather than those of Encephalitozoon. We feel, however, that further studies on Encephalitozoon should be undertaken in order to establish more fully its generic characteristics and status, and the features which distinguish it from Toxoplasma.

The histologic character of Toxoplasma infection has been carefully studied in rabbits and mice by Levaditi and his co-workers²⁴ and in the guinea pig briefly by Mooser²⁵ and in detail by Sabin and Olitsky.¹⁶ The lesions described are focal necroses and indistinguishable from the lesions described in the case reported in this paper.

Specific Identification of the Parasite.—Many species of Toxoplasma have been described in different hosts, but all of these have been morphologically identical. Such specific names should not be accepted without further evidence that differences exist, since specificity for a certain host is conspicuously lacking among Toxoplasma. Toxoplasma gondii, for example, will infect a variety of mammals (mouse, rabbit, guinea pig) as well as various birds (pigeon, Java sparrow).

Chatton and Blanc¹² reviewed the problem of the specific designation of strains of Toxoplasma and concluded that the majority of the described species would be found to represent simple races. Subsequent investigations have upheld their unitarian view concerning the species of Toxoplasma; in particular, cross immunity between strains isolated from a human subject and a lower animal appears to be complete.²⁶

The strain of Toxoplasma observed in the case described in this paper does not vary from descriptions of the type species, *T. gondii*, in any important particular. The question of its identity with this organism must be left unanswered.

23. It is stated that the two parasites show different staining affinities when the Mann method is employed²² and that Toxoplasma occurs within "neurones," while *E. cuniculi* has not yet been observed in that situation (Levaditi and co-workers^{24a}).

24. Levaditi, C.; Sanchis-Bayarri, V.; Lépine, P., and Schoen, R.: (a) *Ann. Inst. Pasteur* **43**:673 and (b) 1063, 1929.

25. Mooser, H.: *J. Infect. Dis.* **44**:186, 1929.

26. Wolf and others.⁹ Sabin.¹⁷

Question of Inapparent Infection.—It will be noted in table 1 that the infected children evinced symptoms of their infection shortly after birth. This has naturally suggested the possibility of prenatal infection. Such a view of course assumes that the parents themselves were infected with *Toxoplasma*.

Unfortunately, in the published records concerning these infantile *Toxoplasma* infections, no account has been found of an investigation of the parents for toxoplasmosis. Undoubtedly other cases of infantile toxoplasmosis will occur, and it is to be hoped that the opportunity for the demonstration of latent parental infection with *Toxoplasma* will be fully exploited.

The mechanism whereby the infants were infected has not been elucidated. Although transplacental transmission has been assumed, it should be mentioned that comparative studies of *Toxoplasma* infections in animals suggest that other paths of infection might be followed. Mesnil and Sarrailhé²⁷ showed that *T. gondii* may be found in the oviducts, uterus and vagina of an infected animal. Thus infection could take place before placental formation, after placental formation but through the fetal envelopes, or during passage of the infant through the birth canal.

It is known that animals artificially infected with *Toxoplasma* may show no symptoms and may become immune to reinoculation.²⁸ Yet such animals continue to harbor virulent organisms, capable of causing fatal infections in other animals.^{24a} Such asymptomatic or latent infections may occur in man. Latent infections are in general difficult of recognition and require indirect means of demonstration. Thus an asymptomatic *Toxoplasma* infection might easily be overlooked unless some rather unusual circumstance calls attention to it. One such circumstance seems to be the giving birth to a child infected with *Toxoplasma*. Another possible source of information is afforded by the fact that latent infections are known to become patent during the evolution of some second, added infection. It is quite possible that the infection reported in this paper was latent toxoplasmosis which became active during the concomitant evolution of the bartonellosis.

The present case, with those collected from the literature, makes a total of only 7 cases of toxoplasmosis.²⁹ The true incidence is probably much higher than this figure indicates, since human *Toxoplasma* infections, both apparent and inapparent, have undoubtedly escaped detection. Differential diagnostic features (with respect to *Trypanosoma cruzi*, *Sarcocystis* and other organisms) have been given.

27. Mesnil, F., and Sarrailhé, A.: *Compt. rend. Soc. de biol.* **74**:1325, 1913.

28. Laveran, A., and Marullaz, M.: *Bull. Soc. path. exot.* **6**:249, 1913.

29. R. G. Archibald and B. Susu (*Tr. Roy. Soc. Trop. Med. & Hyg.* **17**:482, 1924) found a sporozoon, which was not classified further, in spleen films. The parasites may have been *Toxoplasma*. If so, their large size might afford morphologic grounds for placing them in a distinct species.

Four of the recorded cases have been found in the United States, where toxoplasmosis has been frequently reported in animals. Although rigid proof is lacking, it appears likely that forms of *Toxoplasma* found in animals may be infective for man, since those isolated from any one host are commonly pathogenic for animals of distantly related species.

METHODS OF DIAGNOSIS IN MAN

The clinical features of the 6 earlier reported cases of *Toxoplasma* infection in infants and of the case reported here have been summarized in table 1. In suspected cases the diagnosis may be made by finding the organisms in smears or sections, by animal inoculation, by culture methods or by immunologic tests.

Direct demonstration of the organisms in smears or sections is the method of greatest value. Organisms are probably rare in the circulating blood, but they have been found in the sediment of cerebrospinal fluid in considerable numbers. Other body fluids, exudates and tissues should not be neglected, since the available evidence indicates that almost any tissue may be infected. For reasons brought out earlier in this paper, particular attention should be paid to vaginal exudates.

Animal inoculation is a valuable method but should be utilized with full knowledge of the occurrence of spontaneous infection in apparently normal animals. The possibility that a latent infection may be made patent by the inoculation must also not be overlooked. Intracranial, intravenous, intraperitoneal and subcutaneous injections should be made.

Growth of *Toxoplasma* on the developing chick embryo has been described,^{24a} and it is conceivable that this method might be of value in demonstrating infections which cannot be detected by direct examination.

Sabin and Olitsky¹⁶ and Sabin¹⁷ have developed a rabbit skin protection test, based on the protective action of immune monkey serum against the inoculation of virulent strains of *Toxoplasma*. It is possible that latent infections in man might be diagnosed by utilizing this method.

NOTES ON AN EARLIER RECORDED CASE OF INFANTILE TOXOPLASMOSIS

Dr. Arthur T. Hertig enabled the authors to study sections representing an additional case of human *Toxoplasma* infection.

A report concerning this case appeared in 1934, at which time it was felt that the protozoon detected in the tissues was best classified as *Sarcocystis*, although certain atypical features were clearly indicated.³⁰ In view of the recent increase in knowledge concerning *Toxoplasma* infections, Dr. Hertig has placed his material at our disposal for a review of this case. From careful study of the available tissue and from application of the morphologic criteria described in an earlier paragraph, it appears that the protozoon involved is actually *Toxoplasma*.

30. Hertig, A. T.: *Am. J. Path.* **10**:413, 1934.

The infant was born in Massachusetts after a pregnancy of seven months and died twenty-five days later. No hydrocephalus and no nervous or ocular symptoms were noted during life. At the postmortem examination cultures were made from peritoneal fluid and from the content of brain abscesses. From these, growths of *Bacillus coli*, *Staphylococcus albus* and *Staphylococcus aureus* were obtained.

Examination of tissue sections revealed multiple inflammatory and necrotic foci of bacterial origin in the brain, about the umbilical vessels and elsewhere. The lungs showed terminal bronchopneumonia. The bacterial infections were presumably the cause of death.

In sections of the brain, spinal cord, lungs, adrenal and heart, *Toxoplasma* was also found. The latter was identified by its morphologic properties and by its occurrence as aggregates within the cytoplasm of cells as well as extracellularly. In decided contrast to the preceding cases was the rarity of necrotic and inflammatory changes in the host tissue immediately surrounding the protozoon.

SUMMARY

An unusual fatal generalized infection with a protozoon identified as *Toxoplasma* is reported. The parasite and the lesions produced by it are described in detail. Its identification and differential diagnosis and the methods for its recognition in human tissues are discussed. A review of the literature indicates that this is the first authentic case of toxoplasmosis to be reported in an adult, although 5 cases of predominantly cerebral infection have previously been reported in newly born infants. Possible methods of infection of infants, prenatally and during passage through the birth canal, are discussed.

An additional *Toxoplasma* infection in a young American infant is noted. This case had previously been reported as one of atypical *Sarcocystis* infection. It appears probable that *Toxoplasma* infection may not be excessively rare, particularly in the United States.

PRIMARY CARCINOMA IN THE NEGRO
ANATOMIC DISTRIBUTION OF THREE HUNDRED CASES

WILLIAM S. QUINLAND, M.D.

AND

J. R. CUFF, M.D.

NASHVILLE, TENN.

While social differences in cancer have long been recognized, few studies have been made of its occurrence in Negroes. Consequently, the observations presented here, which are based on 300 cases, all verified by pathologic examination, may be of definite value.

INCIDENCE

In a collection of 6,176 specimens surgically removed from Negro patients in our hospital, 264, or 4.27 per cent, proved to be carcinomatous. Among the 813 cases in which autopsies have been made there were 61 cases of carcinoma, a percentage of 7.5. Although 325 specimens of cancer have been studied, the actual number of cases represented is 300. The additional 25 specimens resulted from handling certain tumors more than once, either in biopsy and again after radical operation or after operation and again at autopsy.

PRIMARY ANATOMIC DISTRIBUTION AND CHARACTERIZATION

Primary carcinoma occurred in twenty-six different sites, with the squamous cell type predominating. This type of cell is widely distributed normally, and it occurs also as a result of metaplastic conversion of other types of epithelium into the squamous variety. Such metaplasia is not infrequently met in certain pathologic conditions—for example, vitamin A deficiency¹ and certain chronic inflammatory processes.

The most frequent site of cancer in this series was the uterus. Uterine carcinoma constituted 37 per cent of the collection, and carcinoma of the cervix made up 88.3 per cent of the uterine group. The gross manifestations appeared either as ulcerative necrosis of the portio vaginalis or as diffuse induration of the entire cervix or as a fungating, cauliflower-like mass that protruded into the vagina. Structurally these were of the squamous cell variety and differed in appearance according to the rapidity of growth and also according to the age of the patient.

From the Pathological Laboratory, George W. Hubbard Hospital, Meharry Medical College.

1. Blackfan, K. D., and Wolbach, S. B.: *J. Pediat.* **3**:679, 1933.

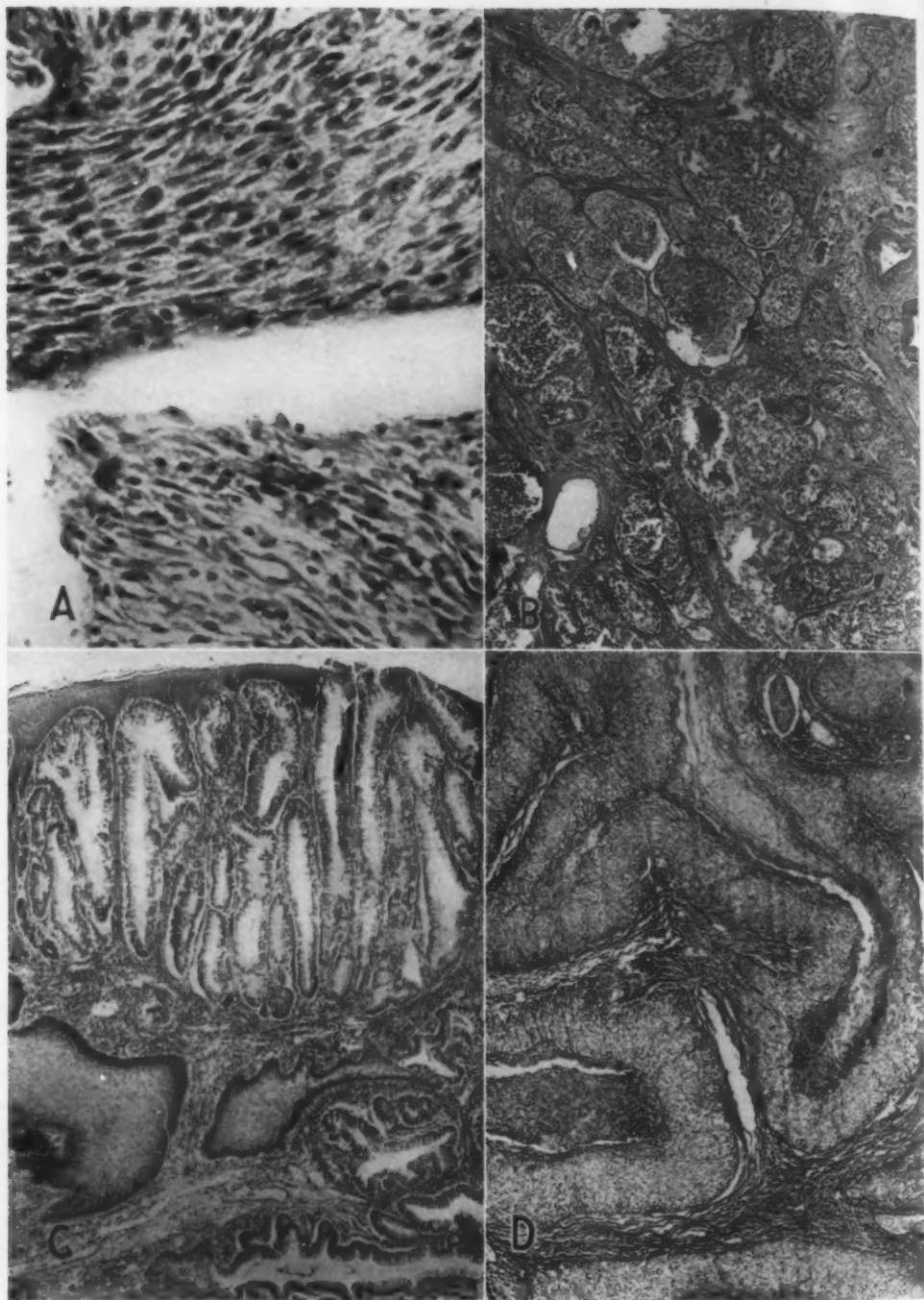


Fig. 1.—*A*, squamous cell carcinoma of the cervix uteri, of the plexiform type. Note the pleomorphism of cells, which show deeply basic-staining nuclei and innumerable mitoses. The stroma is relatively scant.

B, squamous cell carcinoma of the cervix uteri, from a patient aged 22. Note the many concentrically grouped epithelial cells and central disintegration of pearls.

C, adenocarcinoma of the cervix uteri. Note the regional destruction of stratified squamous epithelium by the tumor, also the hyperplasia and keratinization of the remaining stratified epithelium. Glands of columnar epithelium with clear cytoplasm are active in the secretion of mucus.

D, photomicrograph of the acanthoma of the thyroid shown in figure 5. Note the large variegated loops of stratified squamous epithelium with small nuclei and clear cytoplasm that more especially characterize the tumor. The intervening connective tissue stroma is proportionate.

The more rapidly growing type was found usually in young persons. Such tumors were composed of anaplastic epithelium, deficient in pearls and showing innumerable mitoses in an almost negligible stroma (fig. 1 *A*). The slowly growing type, found more often in older patients, was of large flat epithelial cells with pearl formation. This particular



Fig. 2.—Adenocarcinoma of the corpus uteri; weight, 735 Gm. Note the increased size and thickness of the organ and the scattered thick-walled blood vessels, also the extensive necrosis of the cavity and cervix.

feature was observed in a relatively young patient (fig. 1 *B*). The indurated type with much stroma showed varying degrees of epithelial infiltration in the form of scattered nests and cords. Less frequently the cervix presented an adenocarcinomatous arrangement, in which the tumor, arising from the glands of Naboth, invaded the cervical canal or extended outward to destroy the stratified squamous epithelium of the

portio vaginalis (fig. 1 C). The corpus, affected in 11.7 per cent of the cases, showed characteristically adenocarcinoma, which by extension may, although rarely, invade the cervix and even protrude into the vagina so as to resemble grossly a primary cervical lesion. More frequently this type of tumor invades the entire thickness of the uterine wall (fig. 2) and sometimes the bladder and rectum as well.



Fig. 3.—Ribs showing regional bulging and hemorrhagic necrosis consequent to metastasis of carcinoma from the prostate.

Twenty-two per cent of the cancers arose in breasts. The common types were all represented. There was none of the so-called borderline tumors, such as the one reported by Bloodgood,² in which mastitis was

2. Bloodgood, J. C.: *Ann. Surg.* **93**:247, 1931.

confused with carcinoma. Our youngest patient, aged 19, had a painful enlargement of the left breast that had progressed for one year without evidence of axillary involvement. A clinical diagnosis of adenoma was made chiefly on the basis of age and absence of axillary metastasis. The mass when excised was found to be a scirrhous carcinoma.

Ten and three-tenths per cent of the cancers occurred in prostates. Most of these tumors were large; a few of small size were diagnosed at



Fig. 4.—Stomach showing carcinoma of the pylorus with a large eccentric perforation. The patient died of metastasis eighteen months after resection of this lesion.

autopsy. Rarely there may be no obstruction, and the tumor may be barely large enough to arouse suspicion on palpation, as was the case of one in this collection that had metastasized to many bones of the body, an observation later confirmed at autopsy (fig. 3).

The pancreas was carcinomatous in 10 Negroes (5 males and 5 females). In most of the patients the growth was characteristically

adenocarcinomatous and desmoplastic, and occurred in the head of the organ. The patients were between the ages of 31 and 65.

The stomach was the site in remarkably few cases if one considers the frequency with which the digestive tract is affected in males. The gastric tumors, however, presented a variety of morphologic appear-



Fig. 5.—Acanthoma of the thyroid, 16 by 14 by 11.5 cm.; weight, 1,000 Gm. Note the central cystic degeneration. On the surface is a corona of exuberant granulation tissue with a discharging central sinus. A collar of surrounding skin is intact. The patient died of recurrence and generalized metastasis one hundred and twenty days after operation.

ances; there was the encircling infiltrating type at the pylorus; there was the fungating type in the corpus, and there was the ulcerative perforating type in the anterior wall near the lesser curvature; the latter,

shown in figure 4, did not produce general peritonitis, as might be supposed, because of the persistence of limiting serosa, which was backed by adherent omentum. The 2 examples of so-called colloid carcinoma recorded were encountered in males.

All the tumors of the thyroid were found in females between the ages of 24 and 71. The type adenocarcinoma prevailed. One rare cancer of tremendous size, weighing 1,000 Gm. (fig. 5), had been present as a large mass for thirty-nine years in a patient aged 56 years. It showed, when removed, the physical structure of a mixed tumor with fibroblastic stroma and cartilage, also an abundance of epithelial cells in stratified squamous loops and cuboidal epithelium in adenocarcinomatous arrangement. The squamous epithelium more specifically characterized the tumor, which was of woody hardness. The acanthomatous features and the duration of the tumor gave one the impression that the cancer in all probability developed from misplaced squamous epithelium, supplemented by a primary cystic adenoma that later had become cancerous (fig. 1 *D*). Ewing³ supported the possibility of such destructive acanthomas and mentioned Langhans' belief that they may arise from the thyroglossal duct and its pyramidal process.

All carcinomas of the tongue occurred in females and were epidermoid in type. This is contrary to the usual sex incidence, and from the histories the occurrence may be attributed to persistent irritation by carious teeth and habitual "snuff dipping."

The group of renal tumors was comprised of 4 examples of adrenal cell carcinoma (from patients 43, 48, 49 and 60 years of age), 2 of the squamous cell type and 1 tumor of renal cell origin.

The few cancerous growths encountered in livers were an adenocarcinoma of bile duct origin, in a woman aged 44, and 3 hepatomas that showed varying degrees of necrosis, in patients of 35, 53 and 65 years, respectively. Atrophic cirrhosis, regarded as a contributory factor in cancer of the liver, was present in a moderate degree in 2 patients; likewise jaundice was present as a complication. The bile duct cancer listed was an incidental observation at autopsy.

There were 8 carcinomas of skin, 3 of which were of the basal cell type and occurred in women aged 54, 60 and 63 years, respectively. The remaining 5 were of the epidermoid type. These findings are significant, as it is a generally accepted belief that cancer of the skin is relatively rare in Negroes.

3. Ewing, J.: *Neoplastic Diseases*, Philadelphia, W. B. Saunders Company, 1919.

A comprehensive grouping of all affected organs is given in tables 1 and 2, which show the exact number, regional distribution, age-sex incidence and characterization of the primary carcinomas in this collection.

TABLE 1.—*Anatomic Distribution of Carcinoma in Negroes and Number of Cases*

Organ	Number	Organ	Number
Uterus, cervix.....	99	Skin.....	8
corpus.....	13	Thyroid.....	7
Breast.....	64	Penis.....	5
Prostate.....	31	Tongue.....	4
Pancreas.....	10	Urinary bladder.....	4
Jaw.....	10	Nose.....	3
Stomach.....	8	Lip.....	2
Rectum.....	8	Esophagus.....	2
Kidney.....	7	Pharynx.....	2
Liver.....	4	Gallbladder.....	2
Clitoris.....	1	Testis.....	1
Ullum.....	1	Urethra.....	1
Cecum.....	1	Ovary.....	1
Colon (splenic flexure).....	1		
		Total.....	300

TABLE 2.—*Incidence of Carcinoma in Negroes According to Sex and Age*

Sex	Type of Cancer	Total Num- ber of Cases	Cases at Given Age														Un- known
			30 to 25	36 to 31	41 to 36	46 to 41	51 to 46	56 to 51	61 to 56	66 to 61	71 to 66	76 to 71	81 to 76	85 to 81			
♀	Adenocarcinoma.....	49	2	2	3	7	6	7	4	5	4	1	2		
♂	Adenocarcinoma.....	48	1	..	4	..	5	4	3	7	4	9	4	..	1		
															(106 yr.) 1		
♀	Carcinoma simplex.....	7	1	..	3	..	1	..	1	1		
♂	Carcinoma simplex.....	7	1	1	1	1	..	1	1		
♀	Bile duct carcinoma.....	1	1		
♀	Hepatoma.....	1	1		
♂	Hepatoma.....	2	1	1		
♀	Scirrhus carcinoma.....	39	1	..	2	4	5	8	6	5	4	2	2		
			19 yr.														
♂	Scirrhus carcinoma.....	2	1	1		
♀	Renal cell carcinoma.....	1	1		
♀	Adrenal cell carcinoma.....	2	1	1		
♂	Adrenal cell carcinoma.....	2		
♀	Squamous cell carcinoma	111	9	8	10	19	11	15	8	3	8	3	..	1	1		
♂	Squamous cell carcinoma	19	1	..	1	2	1	3	4	3	..	1	1	..	2		
♀	Medullary carcinoma.....	4	..	1	2	1		
♂	Colloid carcinoma.....	2	1	1		
♀	Basal cell carcinoma.....	3	1	1	1		
♀	Total number of cases of carcinoma	218	12	11	15	31	26	32	23	16	18	10	1	1	1		
♂	Total number of cases of carcinoma	82	2	..	6	2	8	10	8	11	6	11	5	..	2		

COMMENT

That cancer occurs more frequently in females than in males is a well established fact which is supported by the findings in this series, which show 218 females affected, 72.6 per cent of the total group. The ages of the female patients ranged from 19 to 85 years, with the highest incidence of carcinoma between 46 and 50. The uterus was involved in

the greatest number, the cervix bearing the brunt of the involvement. These observations are confirmed in Hoffman's ⁴ statement that cancers of the female generative organs are excessive at all ages among Negro women; he further showed by comparison that the incidence for all ages is greater in Negro women (38.4 per cent) than in white women (24.9 per cent). Additional statistical reports from others, Dublin and Lotka ⁵ and Holmes, ⁶ support this view, with but slight numerical variations. Holmes attributed the general increase to the frequency with which carcinoma of the cervix occurs in young Negro women as compared with white women. Dublin and Lotka concurred and mentioned the rarity of such occurrence in Jewesses.

The mooted question is sometimes raised as to what extent primitive habits among Negroes and civilization influence the development of cancer. There seemingly is still no unanimity of opinion, even though individual impressions may be formed on the basis of personal experience. Vint ⁷ found only 8 cases of primary carcinoma of the liver in 11,000 autopsies on the natives of Kehya, Africa, while Strachan ⁸ found 37 such cases in a total of 73 cases of carcinoma among the Bantu races of South Africa, and 277 instances of carcinoma among 2,378 cases of malignant disease in the natives of Kenya. In the United States comparative racial reports by Dublin and Lotka showed that in the year 1935 malignant tumors of the liver and gallbladder were definitely higher among the white persons of each sex; the morbidity rate of liver and gallbladder cancer among policyholders was 22 per cent higher among white females than among white males, and 5 per cent higher among Negro females than among Negro males. The death rate from cancer of all organs was greater among white persons, and the prostate and uterus were the only two organs in which the occurrence of cancer was greater among Negroes than among white persons.

SUMMARY

In 300 cases of carcinoma in Negroes there was involvement of twenty-six different parts of the body. The patients ranged in age from a young woman of 19 to an old man whose age was given as 106. Most of the tumors were found in women between the ages of 46 and 50. In females the organ most frequently involved was the cervix uteri, and the second in frequency was the breast. In males the organ most fre-

4. Hoffman, F. L.: *Am. J. Surg.* **14**:235, 1931.

5. Dublin, L. I., and Lotka, A. J.: *Twenty-Five Years of Health Progress*, New York, Metropolitan Life Insurance Company, 1937.

6. Holmes, S. J.: *Am. J. Cancer* **25**:357, 1935.

7. Vint, F. W.: *Lancet* **2**:628, 1935.

8. Strachan, A. S.: *J. Path. & Bact.* **39**:209, 1934.

quently affected was the prostate and not the digestive tract. The highest incidence in males fell between the ages of 66 and 70. The cases are too few to conclude that such high prostatic incidence is a racial peculiarity, although Dublin and Lotka in four consecutive years of statistical study found this to be a likely possibility.

Squamous cell carcinoma is the commonest type, with adenocarcinoma in second place.

The increase in the number of instances in which cancer is diagnosed in Negroes is made possible by the better preparation to recognize the disease as well as by the greater clinical interest now taken in the Negro people, which is generally becoming more cancer conscious.

RELATION OF ANATOMIC PATTERN TO PATHOLOGIC CONDITIONS OF THE CORONARY ARTERIES

MONROE J. SCHLESINGER, M.D., PH.D.

BOSTON

The location of the main human coronary arteries and the usual distribution of their branches is supposedly a matter of basic, well grounded anatomic knowledge. The branches which are considered most prominent and most constant have been given Latinized descriptive names and thus presumably relegated to oblivion. All experimenters with the coronary artery tree, however, find that its pattern is distinctly not constant. Few systematic studies of the variations and fewer correlations of these variations with human cardiac disease have been made. However, these variations of coronary arteries can be systematized and correlated with the incidence of (1) cardiac infarcts, (2) coronary arteriosclerosis and (3) coronary artery occlusions.

The anatomic data utilized resulted from an attempt to improve and standardize the routine study of pathologic conditions of the human coronary arteries. A new technic was devised, because all previously available methods proved inadequate for visualizing the entire coronary artery tree simultaneously. The older methods fall into four categories: (1) careful, otherwise unaided manual dissection; (2) injection of metal, wax, colloidin and other substances followed by complete digestion of the heart; (3) injection of an opaque medium and clearing of the heart, and (4) injection of a roentgenographically opaque medium and taking a roentgenogram of the heart.

Unaided manual dissection is difficult, time consuming and always incomplete, and its value varies with the care with which it is carried out. Much misinformation based on the results of such incompletely and carelessly performed dissections has crept into the literature. The corrosion or digestion method, brought to a high degree of efficiency by Whitten,¹ gives good anatomic models of the circulation but completely destroys the rest of the heart and most of the pathologic appearances present. Spalteholz'² clearing method yields occasional strikingly beau-

From Harvard Medical School and Beth Israel Hospital.

This investigation was aided by a grant from the Josiah Macy Jr. Foundation.

1. Whitten, M. B.: *Arch. Int. Med.* **42**:846, 1928.

2. Spalteholz, W.: *Die Arterien der Herzwand*, Leipzig, S. Hirzel, 1924.

tiful and instructive permanent preparations, but it is too erratic for routine use.

The technic for roentgenographic visualization of the coronary arteries devised by Gross³ has yielded most information in various hands. It is defective in that the roentgenograms thus prepared must be interpreted stereoscopically. In addition, satisfactory dissection of the injected vessels is almost impossible after the fixation, which is a necessary part of the procedure.

For the past two years all the human hearts available at autopsy have been routinely injected by a modification⁴ of the Gross method. The substitution of lead phosphate agar for Gross's barium sulfate gelatin permitted unrolling of the injected but still unfixed heart. The main coronary arteries were thus flattened out in one plane before the roentgenogram was taken. The necessity for stereoscopic viewing of such roentgenograms was thereby obviated. In these injected but unfixed hearts the injected vessels can easily be completely and carefully dissected out. The method also yields a permanent record of the exact anatomic pattern of the coronary arteries in every heart examined. Also, in every heart all points of coronary arteriosclerosis, occlusion, thrombosis, anastomosis and myocardial infarction or fibrosis can be exposed undistorted and with certainty.

Based on the data thus acquired, descriptions of the development, distribution and significance of the compensatory anastomotic circulation connecting the various branches of the coronary artery system have been published elsewhere (Schlesinger⁴; Blumgart, Schlesinger and Davis⁵). The relationship in individual hearts between these newly developed anastomoses and the presence or absence of coronary arteriosclerosis, of coronary artery occlusions and of cardiac infarcts has been demonstrated. With accumulation of data on a considerable number of hearts thus studied, another relationship between the congenital anatomic pattern of the coronary arteries and these same pathologic changes has also become evident.

In the routine study of 269 consecutive human hearts examined by this method, marked arteriosclerosis, coronary artery occlusions or cardiac infarcts were found absent under the age of 37. Hence the analysis of the relationship of these changes to the anatomic pattern was restricted to the 225 hearts over 35 years of age.

The coronary arteries of a human heart as seen in a roentgenogram thus prepared are shown in figure 1A. Directly in the center of the

3. Gross, L.: *The Blood Supply of the Heart*, New York, Paul B. Hoeber, 1921.

4. Schlesinger, M. J.: *Am. Heart J.* **15**:528, 1938.

5. Blumgart, H. L.; Schlesinger, M. J., and Davis, D.: *Studies of the Relation of the Clinical Manifestations of Angina Pectoris, Coronary Thrombosis, and Myocardial Infarction to the Pathologic Findings*, *Am. Heart J.* **19**:1, 1940.

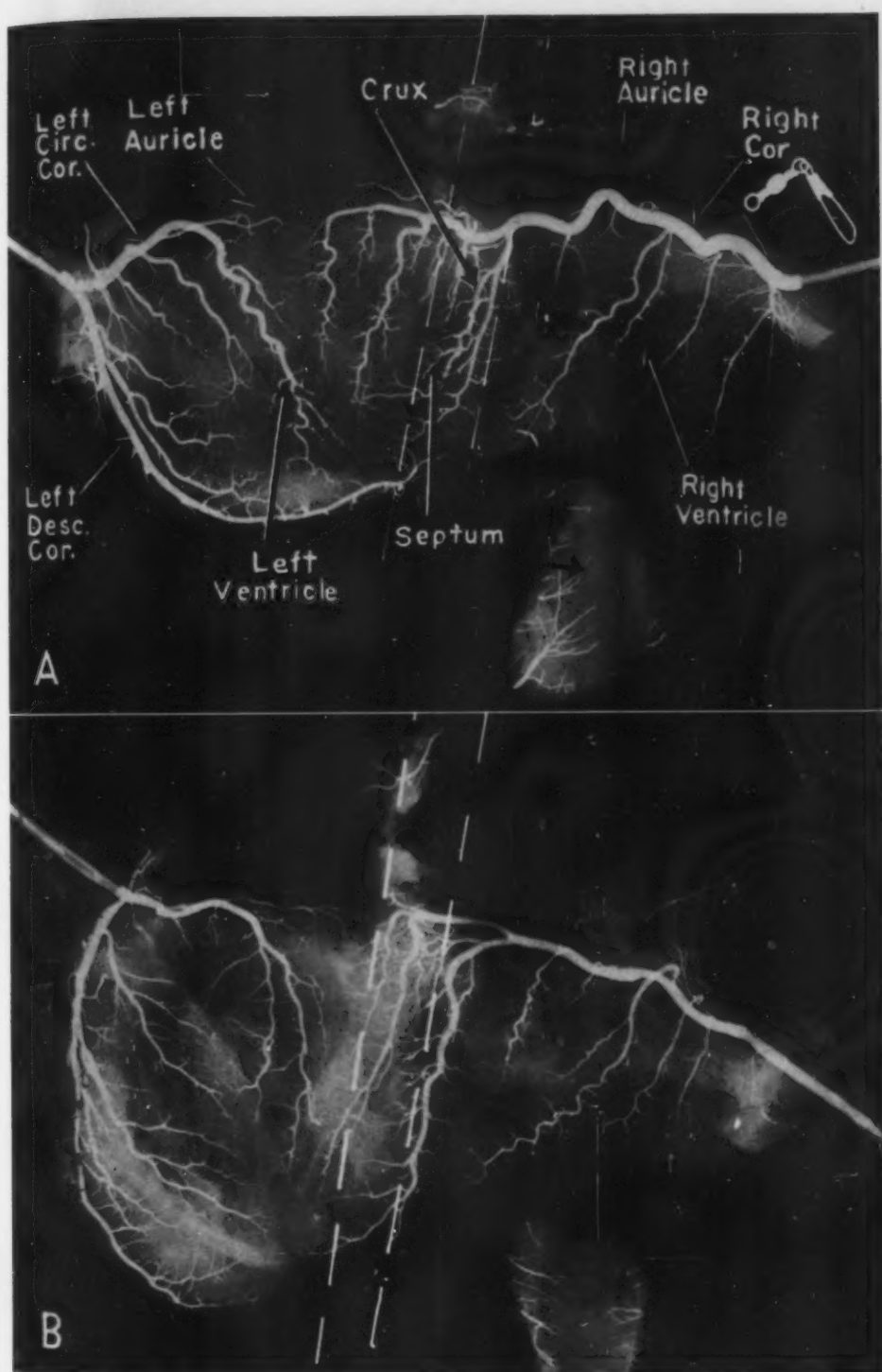


Fig. 1.—*A*, coronary arteries of a human heart of group I, i. e., with the right coronary artery preponderant in the cardiac blood supply. *B*, coronary arteries of a human heart of group II, i. e., with a balanced circulation.

figure is the area called the crux of the heart. This is the region on the posterior pericardial surface of the heart corresponding to the place where the left and right ventricles, the left and right auricles and the interventricular and interauricular septums all meet. Whitten,¹ and Spalteholz² before him, had noted extreme variability in the pattern of the coronary arteries extending to this point. Spalteholz² elaborated diagrams of Bianchi's analysis of these variations in 100 hearts. Adaptations and modifications of Spalteholz' diagrams have been widely published since. Bianchi⁶ had already emphasized the fact that the fundamental variable at the crux of the heart depends on a reciprocal relationship between the length of the left circumflex and of the right coronary arteries. Either one or both of these vessels may extend to the crux of the heart, from which point the so-called posterior descending coronary artery descends. The descending artery or arteries may, therefore, arise from either or both of the main trunks. This variation determines how the blood supply to the heart is apportioned between the two main coronary arteries. The series of 225 hearts over 35 years of age was classified on the basis of this single anatomic variable so closely related to the nourishment of the heart.

In the heart depicted in figure 1 *A*, the right coronary artery supplies blood to a large part of the posterior wall of the left ventricle. In addition, from it arises the posterior descending coronary artery. This heart is a right coronary artery-preponderant heart because this artery supplies all of the right ventricle plus the posterior half of the interventricular septum plus part of the left ventricle. All the hearts of this type, comprising 48 per cent of the series of 225 hearts, have been designated group I hearts.

In contrast to the heart with right coronary artery preponderant, the heart presented in figure 1 *B* has a balanced coronary artery circulation. Each of this heart's two ventricles receives its entire blood supply from the correspondingly named coronary artery. In this type of heart the right coronary artery has no significant branches extending to the posterior half of the left ventricle, and the left circumflex coronary artery has no branches traversing the posterior interventricular sulcus. Here the right coronary artery supplies only the right ventricle plus the posterior half of the interventricular septum, and the left coronary artery supplies the left ventricle plus the anterior part of the interventricular septum. The hearts with this type of balanced circulation, or group II hearts, comprise 34 per cent of the series of 225 hearts.

In the remaining 18 per cent of the human hearts in the series the blood supply is unbalanced in a direction opposite to that in the group I hearts. In these group III hearts with the left coronary artery pre-

6. Bianchi, A.: *Arch. ital. di anat. e di embriol.* 3:87, 1904.

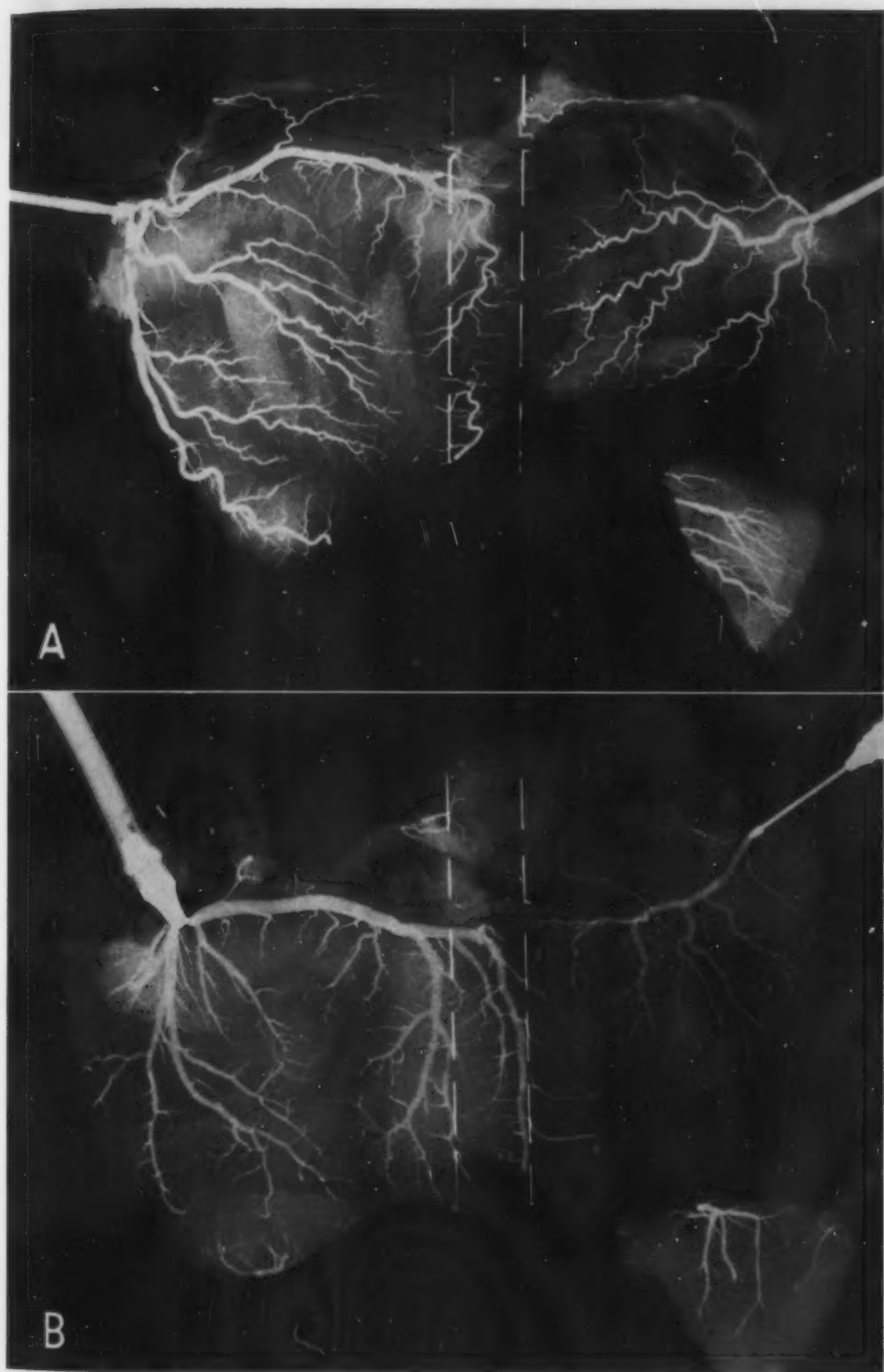


Fig. 2.—*A*, coronary arteries of a human heart of group III, i. e., with the left coronary artery preponderant in the cardiac blood supply. *B*, coronary arteries of a dog's heart. The pattern resembles that of human hearts of group III.

ponderant, the left coronary artery supplies more than the left ventricle and the anterior part of the interventricular septum. There are various degrees of this preponderance of the left coronary artery. In the least obvious form both the right coronary artery and the left circumflex coronary artery extend to the crux of the heart, and both terminate in parallel posterior descending branches. In other hearts the terminal branch of the left circumflex coronary artery constitutes the sole posterior descending coronary artery. Such was the situation in the heart shown in figure 2 *A*. In such a heart the left coronary artery supplies not only the whole of the left ventricle but also the entire interventricular septum both anteriorly and posteriorly. The most exaggerated form of the preponderance of the left coronary artery characterizing the hearts in group III is the reverse of the situation in the group I hearts, in that the left coronary artery there supplies blood not only to all of the left ventricle and the entire interventricular septum but also to a part of the right ventricle.

For brevity of discussion, hearts in which the right coronary artery is preponderant will be referred to hereafter as group I hearts, the hearts with balanced circulation as group II hearts and the hearts with various types of preponderance of the left coronary artery as group III hearts.

The group III hearts, although comprising only 18 per cent of the series of 225 hearts, form a distinct anatomic and physiologic group and also an important pathologic group. Of the three groups of hearts, group III is the most vulnerable to the effects of pathologic changes in the coronary arteries. As pointed out by Piannetto,⁷ the coronary artery pattern of the dog heart is quite constant and generally resembles the least common group III type of human heart. In figure 2 *B* is depicted a roentgenogram of a typical dog heart. This close resemblance of the coronary artery pattern of almost all dogs to the pattern found in group III human hearts necessitates extreme caution in transferring to all human hearts the results and conclusions arrived at by experimental interference with the circulation of the dog heart.

Almost one half of both men's and women's hearts are group I hearts, as shown in line B of table 1. The remaining human hearts, however, are differently distributed in the two sexes. Although in both sexes there are more group II than group III hearts, in women as compared with men there is a disproportionately large number of group II hearts. This sex difference is important because it was found that not only is the group III heart the most vulnerable to the effects of coronary artery disease but the balanced or group II heart is the least vulnerable in this respect.

7. Piannetto, M. B.: *Am. Heart J.* **18**:403, 1939

TABLE 1.—Distribution of Two Hundred and Twenty-Five Human Hearts Over Thirty-Five Years of Age According to Coronary Artery Pattern and Sex (lines A and B); Further Subdivision Showing the Incidence (1) of Myocardial Infarcts (lines C, D, E, F, G); (2) of Coronary Arteriosclerosis (lines H, I, J); (3) of Coronary Artery Occlusions (lines K, L, M)

	Group I				Group II				Group III				Total		
	Hearts with Right Coronary Artery Preponderant in Blood Supply				Hearts with Balanced Circulation				Hearts with Left Coronary Artery Preponderant in Blood Supply						
	Males	Females	Males plus Females		Males	Females	Males plus Females		Males	Females	Males plus Females		Males	Females	Males plus Females
Distribution of Infarcts															
A. Number of hearts.....	68	40	108		47	29	76		30	11	41		145	80	225
B. Percentage of total to each sex.....	47	50	48		32	36	34		21	14	16		100	100	100
Distribution of Coronary Arteriosclerosis															
C. Hearts without infarcts.....	52	34	86		40	29	69		25	10	35		117	73	190
D. Hearts with infarcts.....	16	6	22		7	0	7		5	1	6		28	7	35
E. Percentage of hearts with infarcts.....	23	15	20		15	0	9		17	9	15		19	9	16
F. Hearts with healed infarcts.....	11	3	14		7	0	7		0	0	0		18	3	21
G. Hearts with recent infarcts.....	5	3	8		0	0	0		5	1	6		10	4	14
Distribution of Coronary Artery Occlusion															
H. Hearts with 0 and + arteriosclerosis.....	34	30	64		27	18	45		21	10	31		82	58	140
I. Hearts with 2+ and 3+ arteriosclerosis.....	34	10	44		30	11	41		9	1	10		63	22	85
J. Percentage of hearts with 2+ and 3+ arteriosclerosis	50	25	41		43	38	41		30	9	24		43	27	38
Distribution of Coronary Artery Occlusion															
K. Hearts without occluded coronary arteries.....	43	29	72		34	28	62		18	8	28		96	65	160
L. Hearts with occluded coronary arteries.....	25	11	36		13	1	14		12	3	15		50	15	65
M. Percentage of hearts with occluded coronary arteries	37	27	33		28	3	18		40	27	37		35	19	29

The difference in vulnerability immediately becomes apparent when the incidence of infarcts in the three groups is examined. In line E in table 1 is shown the percentage distribution of the 35 infarcts, both recent and healed, found among the 225 hearts. In the minds of many observers there seems to be some confusion as to where to draw the line between multiple small cardiac infarcts and diffuse myocardial fibrosis, and also between healed and recent infarcts. No heart in the series was called infarcted unless there was present in it a patch at least 1 cm. in diameter in which the myocardial fibers were largely necrotic, missing or replaced by fibrous tissue. The distinction between a healed and a recent infarct was based on an estimation of the age of the infarct relative to the patient's final illness. This estimation was arrived at after a careful gross and microscopic study of the heart. All the recent infarcts were estimated to be of an age equal to or less than the duration of the patient's final illness. Contrariwise, if the age of the infarct was estimated to be greater than the duration of the terminal illness, it was recorded as a healed infarct.

In all three groups of hearts the traditional lower incidence of cardiac infarction in women than in men was confirmed. Not a single infarct was found in the 29 female group II hearts with balanced circulation. The male hearts in this group had proportionately fewer infarcts than the male hearts in either of the other two groups of male hearts, with unbalanced circulation. When the figures for the total incidence of infarcts in the latter two groups are compared, it seems that the group I heart is apparently at a disadvantage as compared with the group III heart. As shown in lines F and G of table 1, the opposite conclusion is reached if the nature of the infarcts is taken into account, i. e., whether they were recent terminal or old healed infarcts.

In the group I hearts two thirds of the infarcts found were healed infarcts antedating the patient's terminal illness. The findings in the group III hearts were in sharp distinction to this evident tendency for an infarct in a group I heart to heal. All six infarcts in the group III hearts were recent infarcts, coincident with the patient's terminal illness. In other words, no person in this series of 41 patients with a group III heart recovered once an infarct formed in his or her heart. The age of these 6 patients at the time of death varied from 37 to 75 years. Equally striking is the observation that all seven infarcts in the group II hearts were healed infarcts. Not only does an infarct seldom develop in a heart with balanced circulation, but when infarction does occur there, it generally heals and does not lead directly to the death of the patient.

Previous intensive studies⁸ of individual infarcted hearts demonstrated that both the formation and the healing of a cardiac infarct were

8. Schlesinger.⁴ Blumgart, Schlesinger and Davis.⁵

closely related to the speed of formation of the occlusions rather than to the number of arteries occluded. Also, invariably, if given the opportunity to develop, a newly established anastomotic circulation took care of the needs of the myocardium after the narrowing or occlusion of any coronary artery branch. Thus, it was hoped to throw light on these peculiarities in the distribution of infarcts in the different groups by studying the incidence of marked arteriosclerosis with narrowing and of coronary artery occlusion in these same three groups of hearts.

In lines H, I, and J of table 1 the series of 225 hearts is classified according to the degree of coronary arteriosclerosis. The coronary arteriosclerosis was graded as none = 0, slight = 1 plus, moderate = 2 plus, and marked = 3 plus. No heart was classified as displaying marked arteriosclerosis unless at the time of dissection there were recorded several significantly and definitely narrowed, but not necessarily completely occluded, coronary branches. Although this is the traditional method of grading arteriosclerosis, it is subject to many sources of error both of observation and of interpretation. In the more recently studied hearts in the series the actual diameters throughout the dissectible lengths of the coronary arteries have been measured and recorded. The number of hearts thus more carefully studied is too small to yield any conclusive figures.

Even the crude method used for the bulk of the series revealed that 50 per cent of group I male hearts over 35 years of age have moderate to marked coronary arteriosclerosis, an incidence definitely higher than in the other two groups. Within each of the three groups the old observation that women have less diffuse coronary arteriosclerosis than men is confirmed. The least difference between the two sexes in this respect is found in the balanced group II hearts. This disproportionately high incidence of severe coronary arteriosclerosis in the 29 group II women's hearts seems paradoxical in view of the complete absence of infarcts in this selected group of hearts. The key to this discrepancy is found in the distribution of complete occlusions of the coronary arteries in the three groups.

The incidence of hearts with complete occlusion of one or more branches of the coronary arteries is indicated in line M in table 1. All the occlusions there recorded were in the three main coronary arteries or primary branches thereof. Fresh occlusions and old occlusions are not listed separately. It should be noted that with the technic used the disclosure of all points of complete occlusion is unequivocal for every vessel in every heart examined. In this series there was uncovered a higher incidence of occlusions in these vessels than was ever before noted, presumably because all the previously recorded figures were based on inadequate methods of study.

Complete occlusion of a coronary artery was found in only 1 of the 29 women's hearts in group II. There is no obvious explanation for this

virtual absence of occlusions in the presence of a relatively high incidence of diffuse coronary arteriosclerosis in this selected group of women's hearts. The absence of infarcts in this group, however, is consistent with the low incidence of complete occlusions. The high incidence of hearts with occluded coronary arteries in group III, 37 per cent, is likewise consistent with the weakness toward infarction exhibited by this group. This figure becomes more significant, although again inexplicable, when it is recalled that group III hearts show less marked general diffuse arteriosclerosis than the other two groups.

Among the 145 hearts from men over 35 years of age were 50 hearts, or 35 per cent, with at least one point of complete occlusion in the coronary artery system. Two or more points of such complete occlusion were present in more than half of these 50 hearts. Nevertheless, coronary artery heart disease was considered a major factor in the death of only about half of these patients with coronary artery occlusion.

TABLE 2.—*Coronary Artery Occlusions in One Hundred and Forty-Five Male Hearts Over Thirty-Five Years of Age*

Age in years.....	35-44	45-54	55-64	65-74	75+
Total number of hearts.....	17	29	49	35	15
Hearts without occlusions.....	14	22	29	22	8
Hearts with occlusions.....	3	7	20	13	7
Percentage of hearts with occlusions.....	17%	24%	41%	37%	47%

In table 2 these 145 male hearts with occluded coronary arteries are further tabulated according to their distribution in age decades. When the scattered occlusions in hearts under 55 years of age are eliminated, there is demonstrated the appallingly high incidence of 40 per cent of coronary artery occlusions in males over 55 years of age.

The similarities rather than the differences between the group I, II and III hearts are emphasized in figures 3 *A* and *B* and 4, which are the same roentgenograms as figures 1 *A* and *B* and 2 *A*. On these roentgenograms have been outlined the peripheries of the infarcts found in the hearts in the respective groups. As near as possible, the portions of the coronary artery trees where occlusions were present have been blacked out. The small numbers placed next to the blacked-out segments indicate the number of occlusions in that portion of the artery.

In all three groups of hearts the infarcts are largely confined to the left ventricle. No region in this ventricle is immune to infarction. It is difficult to say that any one area is more prone to infarction than any other.

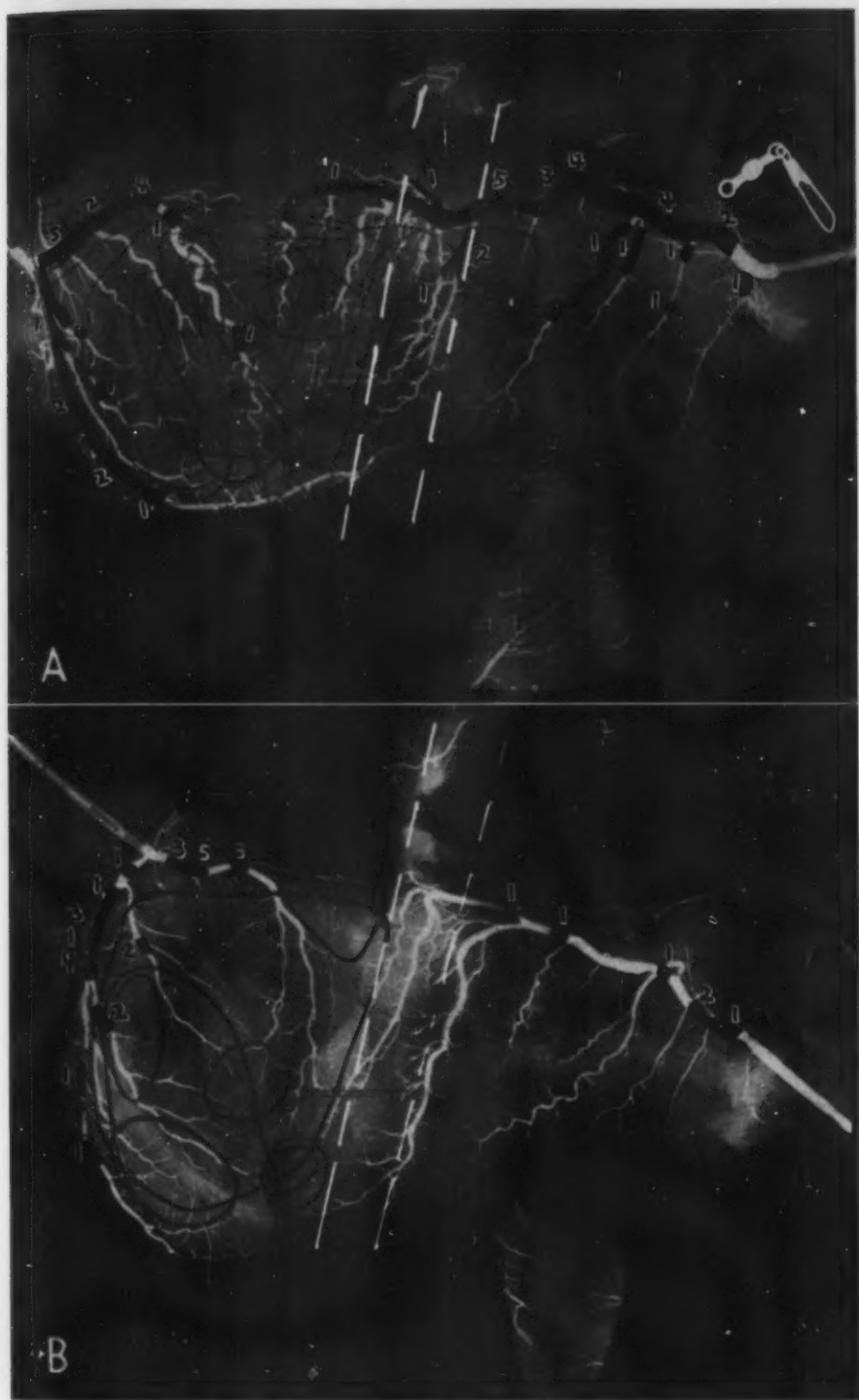


Fig. 3.—*A*, infarcts and coronary artery occlusions in human hearts of group I, with the right coronary artery preponderant in the cardiac blood supply. *B*, infarcts and coronary artery occlusions in human hearts of group II, with a balanced circulation.

The occlusions found are limited to the three main coronary arteries and their primary branches. There is no zone of predilection for these occlusions. They were uncovered throughout the length of the three main coronary arteries. They became fewer in the distal parts of these vessels. In this series the left anterior descending coronary artery does not deserve the name of "the artery of coronary occlusion with sudden death." The frequency and importance of the occlusions in the right coronary artery uncovered in this series have been generally overlooked by others. This will be the subject of another communication.

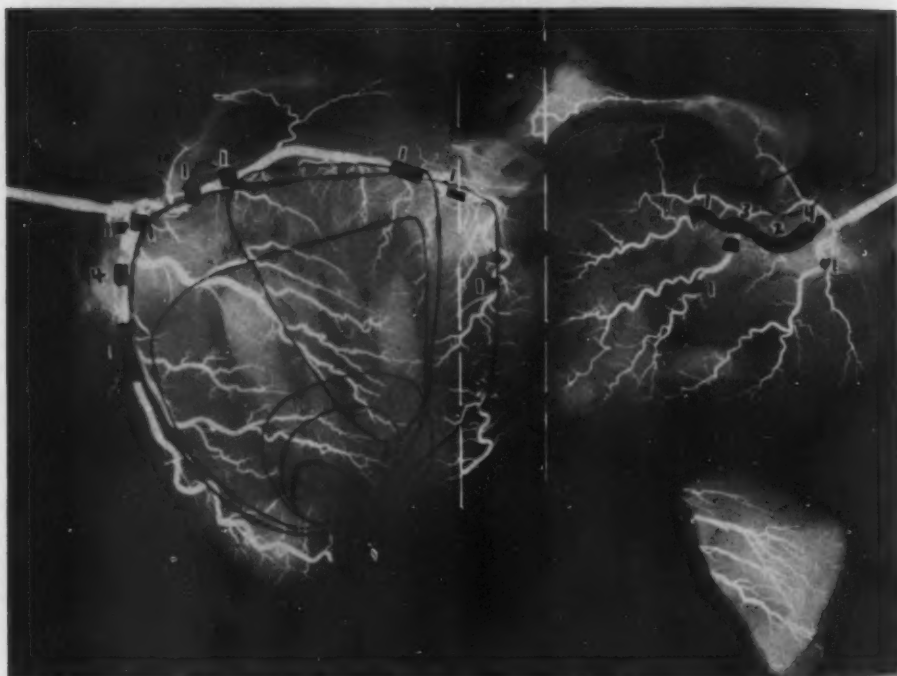


Fig. 4.—Infarcts and coronary artery occlusions in human hearts of group III, with the left coronary artery preponderant in the cardiac blood supply.

COMMENT

In discussing the role of heredity in arteriosclerosis, Osler said that in some people "in the make-up of the machine, bad material was used for the tubing." This may be paraphrased in connection with the coronary arteries: In some people in the make-up of the machine a bad plan was used for the layout. It is a bad plan when the heart originally depends too much on the left coronary artery for its blood supply, as in group III hearts. We⁸ have previously shown that blood coming through compensatory anastomoses from the left coronary artery usually fills the vessels beyond occluded points in either coronary artery, at least in part. When something goes wrong with the blood supply in such group III

hearts, originally depending so largely on the left coronary artery for their nutrition, there is no other reserve system to turn to. The hearts with preponderance of the right coronary artery are better off than these inefficient hearts with preponderance of the left coronary artery, but they do not do as well under adverse conditions as do the hearts with balanced circulation. The layout of a balanced circulation seems to be the best plan for human hearts.

The fact that the layouts for the coronary arteries of dogs' hearts are all like the worst type of layout for the corresponding arteries of the human heart suggests the necessity for a similar study of the coronary artery pattern in different animal species. When the results from such comparative anatomic studies are available, it may be possible to evaluate the evolutionary significance of the several human coronary artery patterns.

The difference of coronary artery patterns may be a hereditary characteristic of the human heart, associated with the well known familial nature of coronary artery disease. Since more than one heart in a family has not been thus studied, there has, as yet, been no opportunity to check this point.

If it were possible to determine the coronary artery pattern in the living patient, it would be an important factor in prognosis. This is not possible as yet. However, the different types of coronary artery patterns may correlate with some other observable factor, such as slightly different electrocardiographic patterns, all within what are now called normal electrocardiograms. This possibility is now under investigation.

Perhaps the different coronary artery patterns correspond to the intrinsic intertwined muscular patterns of the myocardium which Robb⁹ so carefully dissected out. Hearts prepared by the method described should lend themselves well to such a dissection, which must be done on the fresh unfixed heart.

SUMMARY

The coronary artery pattern is not the same in all its details in any two human hearts. These multitudinous variations can be classified into three definitely different, distinctive anatomic and functional groups. In group I, comprising 48 per cent of human hearts, the right coronary artery predominates in the blood supply of the heart; these hearts are intermediate to the other two groups in their reaction to the ravages of coronary arteriosclerosis. In group II, comprising 34 per cent of human hearts, the coronary artery blood supply of the heart is balanced between the right and left coronary arteries; these hearts suffer the least from the effects of coronary arteriosclerosis. In group III, comprising 18 per cent of human hearts, the left coronary artery predominates in the blood supply of the heart; these hearts suffer the most from the effects of coronary arteriosclerosis.

9. Robb, J. S.: *M. Woman's J.* 41:203, 1934.

HYPERSENSITIVITY IN THE ISOLATED RABBIT HEART FOLLOWING INTRAPERICARDIAL SENSITIZATION

BEATRICE CARRIER SEEGAL, M.D.

AND

HERBERT B. WILCOX JR., M.D.

NEW YORK

The *in vitro* test for anaphylaxis devised by Wilcox and Andrus¹ demonstrated that the isolated perfused heart of the sensitized guinea pig reacts in a characteristic and striking manner when small amounts of the specific antigen are added to the perfusion fluid. The heart rate is increased, characteristic electrocardiographic abnormalities occur, and the rate of flow of the perfusion fluid through the coronary vessels is temporarily decreased. These authors also investigated the reaction of hearts removed from sensitized rabbits by the same technic and obtained evidence of a similar response. However, although the rabbits were subjected to a rigorous course of sensitizing injections of antigen according to Grove's² technic, the results were not as regular or as striking as those obtained in guinea pigs. This agrees well with the known difficulty of producing anaphylactic shock in the rabbit.

Seegal and Seegal³ showed that it is possible to produce a local area of sensitization in the rabbit's eye by injecting antigen into the anterior chamber. Placing antigen in this closed cavity, where drainage is relatively slow and antigen bathes local tissues for some period of time,⁴ results in sensitization of the contiguous tissue, which may be demonstrated months later by an intravenous injection of the same antigen. Hyperemia of the iris and conjunctiva, edema and lacrimation result.

It seemed possible that injecting antigen into the pericardial cavity of the rabbit, thus exposing the heart directly to the antigen, might result in local sensitization of this organ. The method of Wilcox and Andrus for studying hypersensitive reactions in the isolated perfused heart seemed well suited for demonstrating such local sensitization. A series of animals was therefore sensitized by intrapericardial injection of

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University.

1. Wilcox, H. B., Jr., and Andrus, E. C.: *J. Exper. Med.* **67**:169, 1938.
Andrus, E. C., and Wilcox, H. B., Jr.: *ibid.* **69**:545, 1939.

2. Grove, E. F.: *J. Immunol.* **23**:101, 1931.

3. Seegal, D., and Seegal, B. C.: *J. Exper. Med.* **54**:249, 1931.

4. Seegal, B. C.; Seegal, D., and Khorazo, D.: *J. Immunol.* **25**:207, 1933.

antigen, and the hearts from these animals, together with hearts from controls sensitized by other routes, were tested.

METHODS

The antigen used was either whole egg white or alum-precipitated egg white. Rabbits weighing 2,000 to 2,500 Gm. were sensitized in one of three ways: (1) by a single intrapericardial injection of the antigen, (2) by a single intraperitoneal injection of the antigen or (3) by intravenous injection of the same quantity of antigen in 10 divided daily doses. The amount of antigen used was 1 cc. of alum-precipitated egg white, or 1 cc. or 0.4 cc. of undiluted egg white.

The alum-precipitated egg white was prepared from a 25 per cent concentration of egg white in physiologic solution of sodium chloride, to which 35 to 50 per cent by volume of 1 per cent alum was added drop by drop with constant shaking. The egg white was kept neutral to litmus paper by the addition of a few drops of normal sodium hydroxide (NaOH). The amounts of alum and sodium hydroxide necessary to obtain a maximum precipitate varied with each sample of egg white used. After the alum-precipitated material had settled the clear supernatant fluid was pipetted off, which resulted in a reduction of the volume by about 50 per cent. No preservative was used, as the material was prepared under sterile precautions and was subsequently proved sterile by culture.

The technic for intrapericardial injection has been described previously.⁵ Briefly, it consists of the following steps: Under ether anesthesia and with aseptic technic an incision is made parallel to the sternum and 0.5 cm. to the left of the sternal border. Retractors are used to spread apart two ribs, exposing the pericardial sac. Since the mediastinal septum of the rabbit is complete, no artificial respiration is necessary, and the operative procedure is simple. The antigen is injected through a fine needle into the pericardial cavity and may be seen bathing the heart. A few small bubbles of air were mixed with the clear egg white in order to visualize better the injected material.

Tests for cardiac sensitization usually were carried out six weeks after the injection of antigen, although in some instances tests were made as early as four weeks and as late as seven weeks. The animals were bled and the serums preserved for titration of antibody. The animals then were put to death by exsanguination and the hearts immediately removed and set up according to the technic of Wilcox and Andrus.¹ Briefly, it consists of the following steps: The heart is placed immediately in chilled Ringer-Locke solution; the vena cavae are ligated; a thread is attached to the right ventricle to record the heart rate, and cannulas are tied into the pulmonary artery and aorta. Ringer-Locke solution at 38 C. and under a pressure of 80 mm. of mercury is then perfused through the aorta; it passes for the greater part through the coronary vessels, and most of this then escapes through the pulmonary artery. This portion of the total inflow is referred to as C flow (coronary flow) and consists of that part of the total coronary flow which has its venous exit through the coronary sinus or via those thebesian vessels which empty into the cavities of the right side of the heart. The rate of this flow is recorded on a kymograph by means of an electric drop recorder.

The remainder of the aortic inflow, i. e., the L, or leakage, flow, escapes through the left auricle and is also measured by means of an electric drop recorder. The L flow consists of that portion of the coronary flow which has its venous exit through those thebesian vessels which empty into the cavities of the left side of the

5. Seegal, D.; Seegal, B. C., and Jost, E. L.: *J. Exper. Med.* **55**:155, 1932.

heart. To it is added the variable but small amount of fluid which escapes by aortic regurgitation. As changes in L flow parallel changes in C flow, only the changes in C flow are recorded in the accompanying table.

Each isolated perfused heart, set up as described, was exposed to a small amount of egg albumin. This was injected through the wall of the rubber tubing into the stream of perfusate about to enter the heart via the aortic cannula.

Precipitins when found in the serums of the animals were present only to a low titer and could not be tested for by the quantitative method. Titration was therefore carried out by the diluted antigen method. The undiluted serums were overlaid with equal quantities of egg white diluted 1:20, 1:100, 1:500 and 1:1,000. Rings were read at the end of thirty minutes at 37 C.; thereafter the contents of the tubes were mixed and allowed to incubate at 37 C. for one hour; precipitates were looked for at the end of twenty-four and forty-eight hours at ice box temperature.

RESULTS

Fifteen animals were sensitized by the injection of alum-precipitated egg white or whole egg white into the pericardial cavity. When the hearts from these animals were perfused with Ringer-Locke solution and 0.05 cc. of 10 per cent egg white was added to the perfusion fluid, 14 of the 15 hearts showed the characteristic hypersensitive or anaphylactic response. The results were the same irrespective of whether alum-precipitated antigen or whole egg white had been used for sensitization. The response consisted of a drop in the rate of flow of the perfusion fluid through the heart. This decreased rate of flow reached its maximum about one and a half minutes after the addition of the antigen to the perfusion fluid. Recovery proceeded rapidly and was accomplished in about two and a half to three and a half minutes. The percental decrease in flow, recorded in the table, varied from 22 to 64 per cent. The upper tracing in the chart shows the kymograph record from the experiment on animal 655. Reading from above down, the heart beat, the C, or coronary, flow collected from the pulmonary artery, the L, or leakage, flow, which escapes through the left auricle, and the time in two second intervals are recorded. It will be seen that both C and L flow decrease so that one and a fourth minutes after the addition of the egg albumin the number of drops is less than one half that at the start of the experiment. The rate of flow then starts to increase. At three minutes the rate of C flow is 70 drops per minute, which is a marked increase over the 38 drops per minute recorded at one and a quarter minutes but is not as rapid as the original rate.⁶ This observation illustrates the fact that in half the animals which showed this hypersensitive response the rate of C flow did not return completely to the original level but reestablished itself at a lower level.

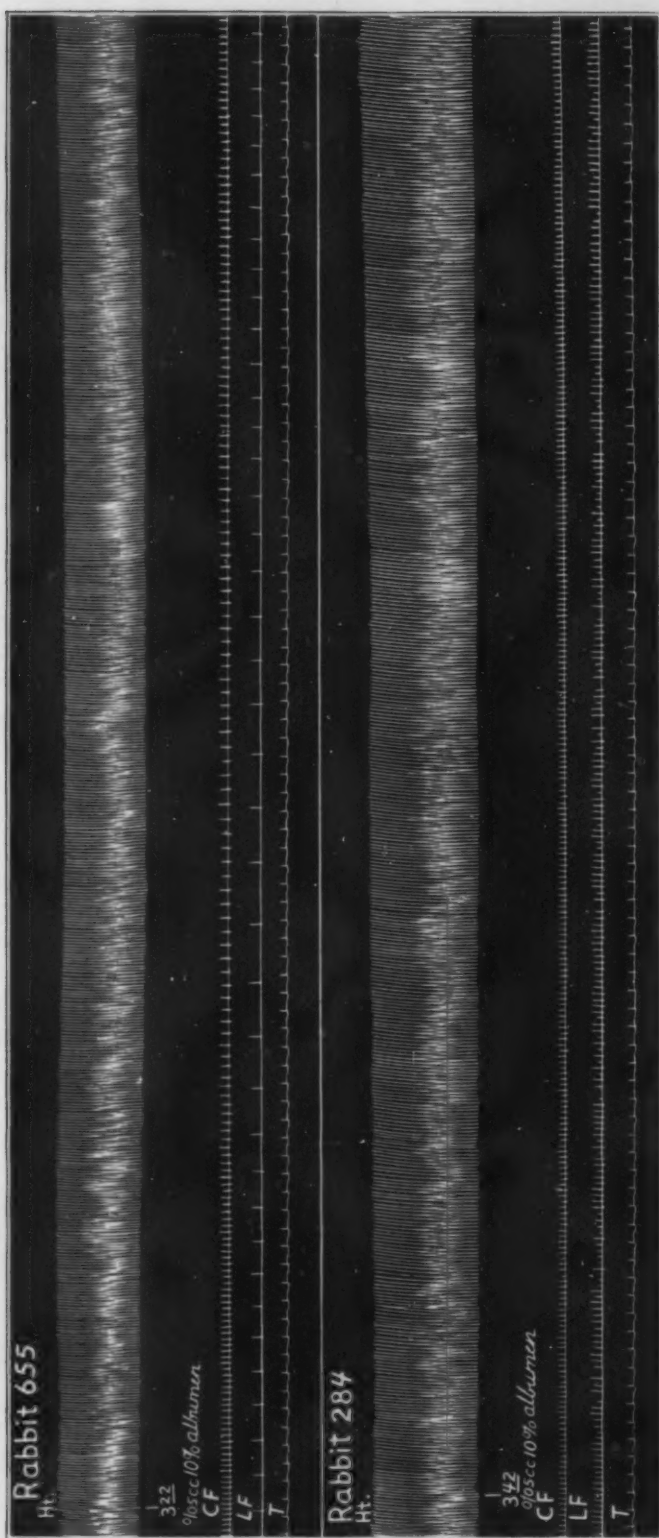
6. The figures given for C flow are obtained by measuring off a six second interval in each one quarter or one half minute of time, counting the number of drops recorded in that six second interval and multiplying by 10.

Effect of the Addition of the Specific Antigen on the Rate of Coronary Flow in the Isolated Perfused Hearts from Rabbits Sensitized Intrapericardially, Intraperitoneally and Intravenously with Alum-Precipitated Egg White or Whole Egg White

Intrapericardial Injection					Intraperitoneal Injection					Intravenous Injections				
C Flow, Drops per Minute					C Flow, Drops per Minute					C Flow, Drops per Minute				
Animal ^a	Before Adding Egg White	After Adding Egg White	Percent- age of Decrease In C Flow†	Precipitin Titer	Animal ^a	Before Adding Egg White	After Adding Egg White	Percent- age of Decrease In C Flow†	Precipitin Titer	Animal ^a	Before Adding Egg White	After Adding Egg White	Percent- age of Decrease In C Flow†	Precipitin Titer
627	130	87	37	Not done	628	210	210	None	Not done	670	83	78	None	Plus 1:1,000
646	145	70	52	Trace 1:100	650	53	47	None	None	675	129	118	None	Plus 1:500
647	58	43	23	Trace 1:100	654	68	62	None	None	676	50	47	None	Plus 1:1,000
653	100	40	51	Trace 1:100	657	29	28	None	None	677	65	23	45	Plus 1:1,000
652	90	70	22	Trace 1:100	656	100	85	15	None	682	105	82	22	Plus 1:500
648	80	59	26	Trace 1:100	661	40	41	None	Trace 1:100	948	105	96	None	Plus 1:500
655	80	38	56	Trace 1:500	669	90	80	None	Trace 1:100					
658	58	21	64	Trace 1:500	666	44	45	None	None					
664	60	41	32	None	685	115	75	35	Trace 1:500					
					686	40	37	None	None					
.....														
585	120	110	None	Trace 1:100	722	132	140	None	None	671	109	99	None	2 plus 1:1,000
286	130	72	45	Trace 1:1,000	687	62	50	None	None	673	66	62	None	Plus 1:500
659	54	38	30	Trace 1:1,000	284	95	92	None	None	674	80	50	None	2 plus 1:1,000
660	88	60	32	Plus 1:100	662	82	77	None	None	681	55	50	None	Plus 1:1,000
667**	108	70	35	None	663	74	40	34	None	683	130	123	None	Plus 1:1,000
668**	53	38	28	Trace 1:100	672**	80	78	None	Trace 1:20	684	22	31	None	Plus 1:500
					678**	128	100	22	Trace 1:500	688	112	80	20	Plus 1:1,000
					679**	130	130	None	None					

^aAnimals above the dotted line were sensitized with 1.0 cc. of alum-precipitated egg white. Animals below the dotted line were sensitized with 1.0 cc. of whole egg white with the exception of those marked with two asterisks, which received 0.4 cc. of egg white.

† A 10 per cent decrease in the rate of C flow is within the normal limits of variation in coronary flow in the perfused rabbit heart. Therefore decreases of 10 per cent or less are recorded as "none."



Upper graph: Heart of rabbit 655, which was given an intrapericardial injection of 1 cc. of alum-precipitated egg white six weeks previously. Note the temporary decrease in C and L flow following the addition of 0.05 cc. of 10 per cent egg white to the perfusion fluid.

Lower graph: Heart of rabbit 284, which was given an intraperitoneal injection of 1 cc. of whole egg white five and one-half weeks previously. No change in rate of C or L flow occurs following the addition of 0.05 cc. of 10 per cent egg white to the perfusion fluid. The heart rate and amplitude do not change in either animal. Time is in two second intervals.

In all cases a second injection of the same amount of antigen was given about five minutes after the anaphylactic reaction. In no case was the coronary flow decreased following the second exposure of the heart to the antigen, indicating desensitization of the heart by the initial injection of antigen. A third injection of double the quantity of antigen, after another five minutes, was also without effect. The anaphylactic response in the rabbit heart was not associated with changes in the rate or amplitude of the heart beat.

The heart from animal 585 failed to show a response to the addition of antigen to the perfusion fluid. The rate of perfusion fell from 120 to 110 drops per minute, but this change in the rate of coronary flow is within the 10 per cent of variation which may occur in normal hearts.

When normal rabbit hearts are perfused with Ringer-Locke solution and 0.05 cc. of 10 per cent whole egg white is added to the perfusion fluid, no change in rate of the C flow greater than 10 per cent occurs. An occasional animal responds to the addition of double this quantity of antigen by either a slight increase or a decrease in the rate of C flow, but within the first half-minute only.

Eighteen rabbits (table) were sensitized by the intraperitoneal injection of alum-precipitated egg white or whole egg white. Fourteen of the hearts from these animals failed to show a slowing of the coronary flow on addition of 0.05 cc. of 10 per cent egg white to the perfusion fluid. One such negative experiment is illustrated by the second kymograph tracing on the chart, for rabbit 284. Four of the animals, 656, 685, 663 and 678, exhibited a hypersensitive response and a drop in rate of C flow which varied from 15 to 35 per cent. In all the hearts subsequent addition of 0.05 cc. and 0.1 cc. of 10 per cent egg white failed to produce a significant change in the rate of flow of the perfusion fluid.

Thirteen animals (table) were sensitized by the intravenous injection of 1 cc. of alum-precipitated egg white or whole egg white, given in ten daily doses of 0.1 cc. each, diluted to a volume of 0.5 cc. with physiologic solution of sodium chloride. Ten of the hearts from these animals failed to react to the addition of 0.05 cc. of 10 per cent egg white to the perfusion fluid. Three of the animals, 677, 682 and 688, showed a decrease in the rate of C flow which varied from 22 to 44 per cent. The subsequent addition of 0.05 cc. or 0.1 cc. of 10 per cent egg white failed to produce a reaction in these hearts.

The results of the determinations of precipitins in the serums obtained at the time the animals were killed are recorded in the table. The animals were put to death four to seven weeks after sensitization, with the greatest frequency in the sixth week. All intervals of time are represented in each of the three groups of animals, so that variation in precipitin titer between groups is not due to length of incubation period.

The amount of precipitate was slight in all cases. Precipitins are recorded in the table as "trace," "1 plus" or "2 plus" at the final dilution of antigen used which gave a precipitate. The highest titers were those of serums from animals that received the antigen by the intravenous route and the lowest were those of serums from animals that received the antigen intraperitoneally. In the former group precipitins were always present in sufficient concentration to give a definite 1 plus or even 2 plus precipitate. In the latter group a trace of precipitin was demonstrable in the serums of only 4 animals, 669, 685, 672 and 678, while the other serums were negative. Serums from 2 animals, 664 and 667, which received the antigen intrapericardially, also failed to show any precipitin. In this intrapericardially prepared group the serum of 1 animal only, 660, had more than a trace of precipitin.

COMMENT

In previous experiments evidence had been adduced to show that the rabbit is a suitable animal for the study of problems of local sensitization. The visible response of hyperemia, edema and lacrimation in the locally sensitized eye of the rabbit which follows intravenous injection of the specific antigen was mentioned previously. The success obtained in this organ was attributed to the fact that when antigen is introduced into the anterior chamber it remains in intimate contact with the surrounding tissues for several days. The pericardial cavity also is a small closed space from which antigen may escape relatively slowly. In previous experiments it was shown⁸ that trypan blue injected into the pericardial cavity diffuses in part through the myocardium. The heart, therefore, is another tissue which can be exposed directly to antigen in high concentration if that antigen is introduced into the pericardial cavity. The method of recording the decrease in rate of coronary flow in the isolated heart, when the specific antigen is added to the perfusate, has made available a delicate physiologic test for the demonstration of a hypersensitive response in the heart. In the experiments described here it was shown that the direct injection of egg white into the pericardial cavity produced sensitization of the heart with great regularity, in 14 of 15 animals. The injection of the same antigen by intraperitoneal or intravenous routes resulted in sensitization of the heart in but 7 of 31 animals. It is thus apparent that the direct exposure of the heart to the antigen by the intrapericardial injection of the antigen results in sensitization of this organ with greater frequency than can be observed with other routes of sensitization.

It is of interest that the hypersensitive response in the perfused heart was obtained at a time when little or no precipitin could be demonstrated

in the circulation. Conversely, the higher titer of precipitin which the intravenously sensitized animals showed was not correlated with increased hypersensitivity of the heart as demonstrated by this technic.

SUMMARY

By utilizing the method of Wilcox and Andrus for demonstrating hypersensitivity of the isolated rabbit heart it was found that a positive result, as indicated by a significant drop in the rate of flow of the perfusate through the coronary vessels, occurred chiefly in animals which had been sensitized *intrapericardially*.

Whereas a positive result occurred in 14 of 15 rabbits sensitized intrapericardially, only 4 of 18 rabbits sensitized intraperitoneally and 3 of 13 rabbits sensitized intravenously showed a similar response.

The antibody titer of the serum was slight in all instances, but the greatest concentration of precipitins occurred in the rabbits sensitized intravenously.

It may be concluded that in the rabbit the intrapericardial injection of egg white produces sensitization of the heart more effectively than either the intraperitoneal or the intravenous injection of this antigen. The degree of sensitization is evidently not correlated with the precipitin titer of the serum.

PATHOLOGIC CHANGES OBSERVED IN HUMAN TISSUES SUBJECTED TO SUBCRITICAL TEMPERATURES

LAWRENCE W. SMITH, M.D.

PHILADELPHIA

During the past three years Dr. Temple Fay and I have been interested in the effect of low temperatures on cancer and embryonal cell growth. Various aspects of these studies have been reported from time to time. Fay and Henny¹ presented certain preliminary clinical observations before the American College of Surgeons, discussing particularly the possible relationship of differences in temperature to the localization of tumors as primary growths and to their metastatic distribution. They further noted the striking relief of pain resulting from the local application of cold (40 to 50 F.) to areas involved in advanced malignant disease and the regular regression in size of the lesion thus treated.

Smith,² at a meeting of the Pathological Society of Philadelphia, reported the results of a prolonged series of observations on the effect of varying temperatures on the growth and development of chick embryos. It was brought out by these studies that exposure of eggs to subcritical temperatures (85 to 95 F.) for a period of forty-eight to ninety-six hours at the outset of incubation—the remainder of the incubation being carried out at optimal levels (100.5 to 101 F.)—would result almost regularly in the production of some developmental malformation, even to actual monster formation. On the other hand, if cell differentiation was permitted to take place by incubation at normal levels during this initial forty-eight to ninety-six hours, the introduction of a period of subcritical temperature later only rarely caused any major developmental catastrophe. Below 90 F. not only was cell growth markedly retarded, but regressive changes were commonly noted, going on to actual necrosis.

At the St. Louis meeting of the American Medical Association, Smith and Fay³ presented a summary of their results in the case of local refrigeration and in that of a general reduction in body temperature, emphasizing in this report the effect of such reduced temperatures

From the Department of Pathology, Temple University School of Medicine, and the Tumor Clinic of Temple University Hospital.

1. Fay, T., and Henny, G. C.: *Surg., Gynec. & Obst.* **66**:512, 1938.
2. Smith, L. W.: *Arch. Path.* **28**:420, 1939.
3. Smith, L. W., and Fay, T.: *J. A. M. A.* **113**:653, 1939.

both clinically and from the pathologic standpoint as demonstrated in the study of serial biopsy specimens in a series of 30 cases of human cancer.

Again, at the International Cancer Congress at Atlantic City, N. J., in the fall of 1939, both Fay⁴ and Smith⁵ presented additional clinical and pathologic data on cases seen since their previous report. And finally, at the Symposium on Temperature held by the American Institute of Physics at New York a month later, Smith and Fay⁶ presented certain clinicopathologic data of general physiologic interest which had accumulated during the course of their other studies.

In the present paper it is hoped to emphasize the histologic studies of these cases, now numbering over one hundred, in which cancers have been subjected to (1) local application of cold, (2) reduction of body temperatures or (3) a combination of both procedures. At the outset it should be stated that almost without exception the cancers which have been observed under these conditions have been in the terminal phases of malignancy, the patients having been admitted to the hospital chiefly because of problems of pain. Each patient voluntarily agreed to serial biopsy studies and to postmortem examination in the event of death. The experimental nature of these studies has been thoroughly understood by the patients and the importance of such repeated histologic examinations realized as being the only sound way in which such reduced temperatures might be evaluated as an adjunctive therapeutic physical agent.

The selection of cases has been made as impartially as possible with a view to studying as many forms of cancer as possible. An attempt has been made to include only cases in which prognosis has indicated at least three to six months of life, as it was felt that an adequate time should be allowed to elapse for the full development of such cytologic changes as might occur. To this end the study has included cases of bone sarcoma, fibrosarcoma, reticulum cell sarcoma, Hodgkin's disease and carcinoma of the antrum, buccal cavity, thyroid, lung, liver, stomach, sigmoid, rectum, kidney, adrenal, testis, prostate, bladder, breast, uterine cervix and skin, as well as glioblastoma multiforme of the brain, a recurrent malignant melanoma of the vulva and acute leukemia.

The majority of these cases, as has been stated, were instances of advanced malignant growth, and the cancerous processes have gone on to their anticipated fatal outcome. However, it is our definite impression

4. Fay, T.: Read before the Section on Surgery, Third International Cancer Congress, Atlantic City, N. J., Sept. 14, 1939, to be published.

5. Smith, L. W., and Fay, T.: Read before the General Section, Third International Cancer Congress, Atlantic City, N. J., Sept. 15, 1939, to be published.

6. Smith, L. W., and Fay, T.: *Am. J. Clin. Path.* **10**:1, 1940.

that in the majority of instances life has been prolonged beyond the period of expectancy and, even more important, that death has been a much less terrible ordeal, through the regular control of pain without the necessity of administering constantly increasing doses of narcotics. Indeed, narcotics have not been required in any case once refrigeration therapy has been instituted. In the selection of such terminal cases we had a twofold purpose: In the first place, we felt that only in such cases in which all the more orthodox surgical and radiation therapy had been received was such an untried procedure justified, and in the second, we deemed it vitally essential that studies of the tissues in general should be made in such cases ultimately at autopsy.

The material for the present study, therefore, can be divided into several categories. There are, first of all, the general autopsy specimens, which, for convenience, can be designated as normal tissues. It is recognized, of course, that other pathologic changes coexist in many such specimens, but only as incidental processes. Some 60 cases in which such specimens were studied are included in this survey.

Secondarily, there are the serial biopsy specimens taken principally from accessible tumor masses subjected to local cold, the return flow of the refrigerant in the circulating system ranging from 40 to 50 F. This does not mean that the temperature of the tumor necessarily approached these levels, as temperatures of the skin recorded by thermocouple showed a differential of from 5 to 25 F., dependent on the character of the local applicator and the accuracy of its application to the surface involved. (Ideally a silver or copper applicator molded to the part would be most effective as a heat conductor.) Many of the patients, in addition to the local refrigeration, were given one or more periods of general reduction of body temperature.

Finally, the material includes the metastatic tumor tissues, which likewise could be studied histologically only post mortem. In many instances, however, serial roentgenologic studies had been made, in some of which actual regression of skeletal metastases was observed, in others of which the process seemed arrested, while in still others no apparent effect on the spread of the lesions could be detected.

Ultimately it is hoped to be able to evaluate statistically the effect of various subcritical temperatures on the course of the many different tumor cell types. It will not be surprising to find striking differences dependent on many factors, especially degrees of cell differentiation. Already it is possible to generalize in a broad way and recognize that the response of sarcoma is much less striking than that of carcinoma. Similarly, the more undifferentiated the carcinoma, the more rapid are the regressive phenomena. Likewise, it is apparent that the local application of cold at 40 to 50 F. is more effective in causing degenerative

changes in tumor cells than is the general reduction of body temperature to levels of 80 to 90 F. But these differences are quantitative rather than qualitative and not particularly significant in this discussion, which deals with the more basic histologic phenomena as a whole.

EFFECTS ON TISSUES IN GENERAL

Let us consider first the effect of reduced body temperatures on the normal structures, as evidenced, first, by the gross pathologic observations at autopsy and, second, by microscopic examination of the various tissues. By and large, it may be stated that in the great majority of cases no significant demonstrable changes can be detected in the various body structures either grossly or microscopically. However, in the course of these studies we have encountered occasional pathologic conditions which may represent potential dangers and complications. These have been observed in some instances following a single period of reduction of body temperature. On the other hand, no changes have occurred in many cases in which four, five and six periods of several days' duration each of such general reduced temperatures have been given. For this reason, it becomes extremely difficult to evaluate the relative importance of such occasional findings.

Heart.—It has been noted both in our own studies and in those reported from the Lenox Hill Hospital⁷ that there is a fairly regular tendency toward reduction in the T wave as shown by electrocardiograms. Not infrequently a prolongation of the preceding complex is observed, suggesting some general myocardial functional disturbance. These changes ordinarily disappear within a day or two after the period of refrigeration has been completed. These observations obviously point to cardiac effects as one of the potential dangers and complications of refrigeration. The autopsy series presented here includes 5 instances in which marked cardiac effects were observed. Various lesser degrees of myocardial degeneration have been encountered in several other cases, but in these the degree of change did not seem to be of clinical significance. In the 5 cases just cited the patient died apparently of cardiac failure within a period of twenty-four to forty-eight hours after general refrigeration. In each of these 5 cases the heart showed at autopsy extensive myocardial degeneration with marked hydropic and fatty changes of the cytoplasm. In 3 of these 5 cases there was found, likewise, associated, rather definite general atherosclerosis of the coronary circulation. This had not been demonstrable clinically, and in no instance was there complete coronary occlusion. It seems reasonable to think of the possibility of diminished blood flow to the heart muscle itself being responsible for

7. At the meeting of the New York Academy of Medicine, Section of Internal Medicine, Feb. 20, 1940.

the development of anoxia with such myocardial change. In the other instances, however, the evidence of coronary disease was absent. There was no appreciable hypertension preceding the induction of reduced body temperature, and there were no symptoms pointing to cardiac changes until the terminal episode of heart failure. In passing, it may be pertinent to point out that no degenerative changes were found in the brain stem to point toward a central type of failure.

In no other case which has been studied post mortem in a series in which the ages of the patients ranged from 18 months to 74 years, has any significant degree of myocardial degeneration been found. The majority of the patients have been in the age group past 40 years, and there have been many other instances of rather marked coronary disease. In fact, 2 patients known to have cardiac disease, 1 with auricular fibrillation, have been carried through periods of induced low body temperature without difficulty. Accordingly, it seems difficult to explain satisfactorily these 5 cases, and I am inclined to the opinion that there must have been some underlying myocardial injury which had not been recognized clinically before observations at lowered temperatures were undertaken.

Lungs.—The relationship of induced temperatures to the development of pneumonia in such patients has been the question which has been most frequently asked of us by physicians who have visited the hospital to learn the technical details of the study. Again, this is a difficult question to answer satisfactorily. Of those subjects who have come to autopsy, an appreciable proportion have shown some evidence of terminal hypostatic bronchopneumonia. This is only to be expected in a group of cases of terminal malignancy. It has been extremely difficult to decide whether the lowered temperature should be considered as a significant factor in this picture, as an analysis of the cases of terminal malignancy in which the refrigeration was not used shows approximately the same incidence of terminal bronchopneumonia. It must be recognized that these patients are in general emaciated, cachectic and anemic, with low resistance to such infection. It is quite possible that the slowed circulation, as shown by Oppenheimer and McCravey⁸ through studies of circulation time, may tend to produce a somewhat greater hypostasis in the lung field. We are convinced that during the period of reduced body temperature practically complete bacteriostasis exists for ordinary pathogenic organisms. It is conceivable that with the return of the temperature to normal, bacterial activity might become implanted in a favorable soil, and thus the reduced temperature might play a minor part in the induction of terminal pneumonia. On the other

8. Oppenheimer, M. J., and McCravey, A.: Circulation Time in Man at Low Temperatures, *Am. J. Physiol.*, to be published.

hand, the majority of the patients go through as many as five and six such periods of induced generalized low temperature without the slightest suggestion of pneumonia.

There is no question but that in cases in which metastatic tumor is present within the lung parenchyma bronchopneumonia develops more frequently. In the series of 26 cases reported at the Lenox Hill symposium by Dixon there were 5 cases of bronchopneumonia in relation to such complicating pulmonary lesions. In none of these was bronchopneumonia found at autopsy. Our own experience has been similar, so that the danger of such secondary bronchopneumonia in this group of cases must be recognized.

Among cases in which a neoplasm has involved the nasopharynx we have seen at least 3 in which there was evidence of inhalation pneumonia developing around necrotic tumor tissue which had been inspired. The evidence that this represents a higher incidence of such a complication in cases of refrigeration is not convincing. However, it is a complication which should be borne in mind, and if treatment of patients with this type of cancer of the upper respiratory or of the digestive tract is undertaken, the problem of maintaining postural drainage should be seriously considered. A similar high incidence of bronchopneumonia is associated with cases of primary pulmonary tumor, and, again, we are inclined to think that no greater incidence of this complication occurs in patients undergoing hibernation than in groups treated by other methods.

It must be recalled that in the presence of such advanced cancer both the patient and the physician are justified in taking certain risks. Palliative treatment of cancer is an expression of defeatism; it is practiced only too frequently by surgeons and radiologists alike. Cancer is fatal unless radically destroyed or removed, and it is our contention that the utilization of reduced body temperature as an adjunct to radical surgical intervention or to radiation therapy is not only desirable but definitely indicated.

In summary, it appears from the study of these pneumonic cases that terminal bronchopneumonia is a complication which may be expected in a considerable proportion of cases of advanced cancer whether or not the patients are subjected to the general reduction of temperature and that in cases in which the lesions are not clinically terminal from the standpoint of malignancy, the danger of such secondary pneumonia is relatively negligible.

Liver.—No pathologic changes have been encountered in the liver which can be pointed to either grossly or microscopically as related to the subjection of the patient to such low temperatures. From clinico-pathologic observations made in chemical studies of the blood the liver

appears to be in a relatively resting state during such episodes of "hibernation." Such changes as have been encountered appear to be entirely incidental to some underlying condition such as chronic cholangitis, chronic passive congestion, cirrhosis or even metastatic neoplasm.

Pancreas.—In 3 instances out of the entire series there was evidence of an escape of pancreatic lipase into the surrounding tissues, producing areas of fat necrosis. In 2 of these cases there was slight associated hemorrhage. In the first of the cases the patient had a very extensive retroperitoneal reticulum cell sarcoma which had extended to the pancreatic region, and which, we believe, was basically responsible for the development of the associated pancreatic condition. In the other 2 cases no such relationship was established. Whether this finding should be considered of any significance, again, is wholly problematic. However, both of the patients had been users of unusually heavy doses of narcotics, and the occurrence of pancreatic disease as a cause of death in chronic narcotism has been commented on not infrequently. In no other case could demonstrable changes be found in the pancreas.

Gastrointestinal Tract.—No uniform change has been encountered in the gastrointestinal tract in relation to reduced body temperature. In 3 or 4 cases in which cold had been applied locally in the rectal region there were noted congestion and edema of the mucosa associated with the formation of small contact areas of ulceration in relation to the applicators. This, in turn, was associated with rather persistent diarrhea for which no adequate explanation could be found. Similar congestion, even to the point of petechial submucous hemorrhages, has been observed in the gastric mucosa in a few instances, which likewise does not seem to have any adequate explanation. Aside from these changes, which have in general not been of clinical significance, nothing noteworthy has been observed.

Kidneys.—No significant pathologic changes have been observed in any instance. In some cases there has been moderate cloudy swelling grossly, with slight granular albuminous degenerative changes in the cytoplasm of the epithelium of collecting tubules microscopically, but whether this can be attributed to the lowered temperatures is highly questionable. There usually have been sufficient other lesions unrelated to the change in body temperature to account for such minor toxic changes. In no instance has there been any evidence of glomerular damage. Azocarmine and Masson stains have been made in several instances in order to appreciate better whether or not any glomerular avascularity or degenerative features could be recognized. None have been observed, nor have any inflammatory changes with leukocytic infiltration or hemorrhage been found. Such changes as have been seen in the glomeruli have always been those to be expected in a group of per-

sons largely of the fifth decade of life or over. In other words, certain vascular phenomena, of either an arterial or an arteriolar character, have been present in many cases. Associated interstitial cellular infiltration of a nonsuppurative character in combination with chronic pyelonephritis has likewise been seen in cases associated particularly with a carcinoma of the bladder or of the prostate which has produced obstruction of a ureter.

Ductless Glands.—(a) *Adrenals:* In 3 or 4 cases there was a sudden unexplained drop in blood pressure, which persisted even after refrigeration was discontinued and from the clinical standpoint seemed to be the most significant feature in explaining the death of the patient. In 3 of these cases there was frank metastatic, relatively extensive involvement of both glands. In 1 case no such metastatic lesions could be demonstrated either grossly or microscopically. Careful histologic study of the adrenal cells showed several features which may or may not be significant. Lipoid depletion was observed with a fair degree of regularity. We felt that this might be more readily explained in relation to the general condition than to any inherent alteration of physiologic activity through the application of low temperatures, but in reviewing the other cases we cannot help thinking that this may be of some significance. In addition, coarse vacuolation with degenerative changes of the cytoplasm of both cortical and medullary cells has been found in these cases, of a degree not previously noted in other cases of the series. It therefore seems possible that not only widespread destruction of the gland as a whole but actual degenerative phenomena may be of clinical significance. As a result, patients in whom any significant drop in blood pressure is observed are now given supportive treatment with an extract of adrenal cortex.

(b) *Pituitary:* The pituitary has shown no changes which seem significant. There has been no disturbance in balance of acidophilic, basophilic or chromophilic cells. No degenerative phenomena involving the cytoplasm of any of the types of cells have been found. The vascular pattern has seemed no more variable than that observed in cases in which there was no refrigeration.

(c) *Thymus:* The thymus has not been observed sufficiently often, because of the general age level of the patients, to be evaluated in respect to any degenerative features. No changes in the glands which might have been considered abnormal were found. In 1 case in which the thymus was involved in an extensive neoplastic process the usual regressive changes of the tumor cells could be seen.

(d) *Gonads:* No changes have been seen in ovaries or testes which were not the site of associated lesions. No demonstrable changes of the interstitial cells or of the ova of the tubules of the testes seem to have

taken place. As in other organs, when the gonad has been the site either of a primary or of a metastatic neoplasm, certain phenomena of degeneration of the tumor cells have been observed.

Brain.—Careful studies have been made of the brains of all subjects coming to autopsy. So far as possible, routine comparable sections have been taken from ten areas in each brain, representing motor and sensory centers of the cortex, the thalamus, the cerebellum and the brain stem, including the several cranial nuclei. None of the usual cytologic changes which are associated with degeneration of the central nervous system have been observed. There has been no alteration in the Nissl granule pattern of the neurons, no change in ratio of nucleus to cytoplasm, no evidence of chromatolysis, no gliosis, including microglial proliferation; there has been no softening and no hemorrhage. In a few instances, particularly in those earlier cases in which sedation with barbiturates was practiced, a slight degree of edema was observed. In the later series of cases this has not been apparent. Indeed, in 1 instance in which a local applicator was inserted through a trephine opening in the skull directly into the brain substance along the margin of a glioblastoma, no degeneration of the normal brain cells was observed at a distance of more than 2 mm. from the applicator.

Summary.—So far as changes in the normal tissues are concerned, it may be stated that prolonged subjection of normal adult differentiated cells to levels of temperature critical for embryonal cells is without significant effect. Neither gross nor microscopic evidence has been found to indicate that such normal cells are influenced harmfully in any way even when subjected almost constantly to temperatures of 40 to 50 F. locally for periods of as long as five months. Indeed, healing can go on uninterruptedly at these levels, as has been seen in a considerable number of cases of ulcerative lesions. So far as complications producing changes of pathologic significance to the subject as a biologic unit are concerned, there have been in this series of cases of terminal carcinoma 5 in which refrigeration was accompanied by unexplained myocardial failure. The possibility, likewise, of terminal bronchopneumonia developing as a complication following the use of reduced body temperatures must be recognized as a risk in cases of such advanced malignancy associated with cachexis and anemia. Whether this incidence is significantly different from the incidence of terminal pneumonia in other cases of advanced malignancy seems extremely doubtful.

CHANGES IN TUMOR TISSUE WITH LOCAL APPLICATION OF COLD

The cell changes which occur in tumors subjected to local application of temperatures of 40 to 50 F. for varying lengths of time have already been described,³ so they need not be gone into in detail at this

time. It is in this group of tumors, however, that the more striking changes are observed, and thus they may be utilized as a yardstick in determining the results of the application of cold either locally or generally. For this reason, an example is herewith presented.

In this series of approximately 100 cases there have been 4 in which the bladder was involved either by a primary tumor of the mucosa of the organ or by an extension from a prostatic neoplasm. The patients were subjected to general reduction of temperature as a means of controlling



Fig. 1.—Patient with applicator in situ and the local refrigerator unit in the background.

pain, with a uniformly satisfactory clinical response. Recognizing that the regressive changes in tumor cells are much more striking and rapid in those instances in which the local application of lower temperatures can be maintained, the bladder was opened suprapubically and marsupialized so that a direct approach with local applicators could be carried out. Figure 1 shows a patient with the applicator in situ and the local refrigerator unit in the background. This consists simply of a pump to circulate a refrigerant (in this instance, ice water) through

a closed system, with the return flow maintained at 40 F. Three photomicrographs (figs. 2, 3 and 4) are presented showing the progressive changes which occurred during a ten week period. Figure 2 represents the original biopsy specimen from a large fungating infiltrative tumor

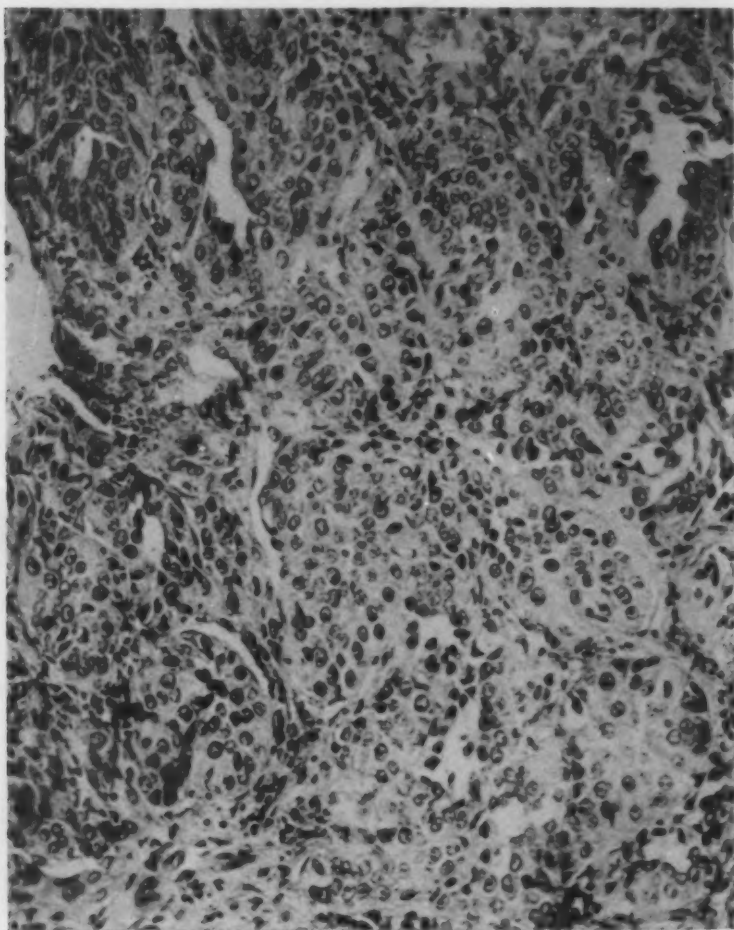


Fig. 2.—Prerefrigeration biopsy specimen from large fungating infiltrative tumor mass in the bladder; approximately $\times 150$. Note the uniform appearance of the cells, with ovoid vesicular nuclei and a characteristic arrangement of chromatin.

mass in the fundus of the bladder. Figure 3 represents a biopsy specimen taken approximately four weeks after the pretherapy specimen was obtained. During this interval the major polypoid mass of tumor tissue had undergone necrosis and sloughed away, leaving an ulcer crater about 2 cm. or slightly more in diameter. The wall of the bladder had become

much less dense. Figure 4 represents a small biopsy specimen obtained from the base of the ulcer crater ten weeks after cold was first applied. At this time the ulcer crater was not more than 1 cm. in diameter and was extremely deep and punched out in appearance. The remainder of

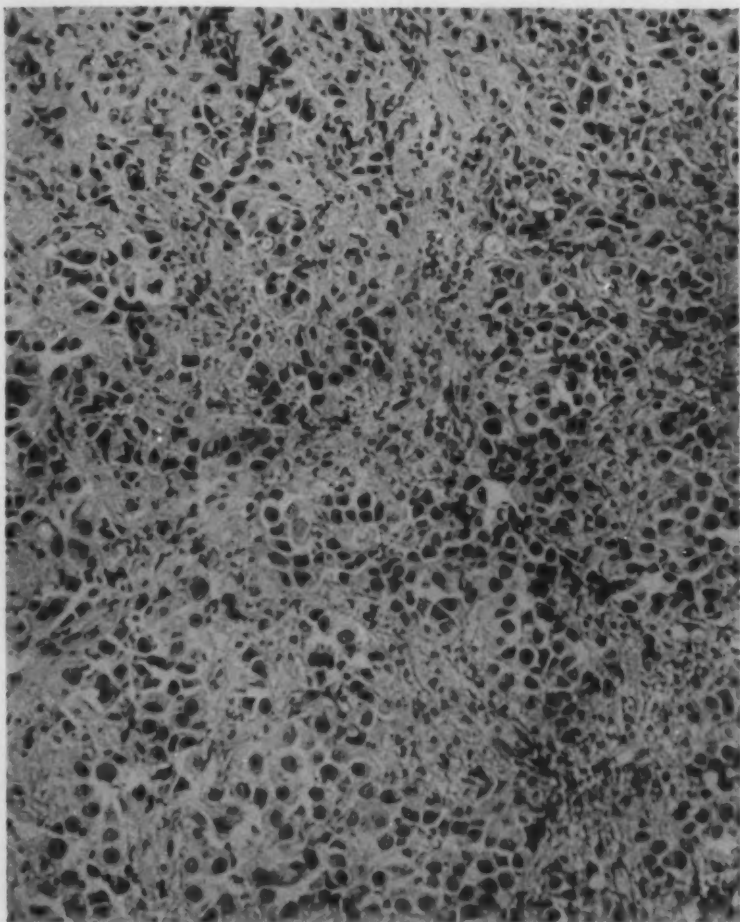


Fig. 3.—Biopsy specimen taken after four weeks of local cold; approximately $\times 150$. The outstanding change at this time is the marked pyknosis and beginning necrosis of the tumor cells.

the bladder wall had become of practically normal texture. Unfortunately, the patient had been suffering from complicating pyelonephritis during the period in which these studies were in progress and died rather unexpectedly and suddenly of this renal complication. The case is presented in this brief fashion simply to illustrate the tissue changes

which may be observed regularly with such local application of cold. It is wished to emphasize in this connection that the normal supportive and differentiated cell structures are not demonstrably affected by this procedure and that normal healing even of large ulcerated surfaces goes

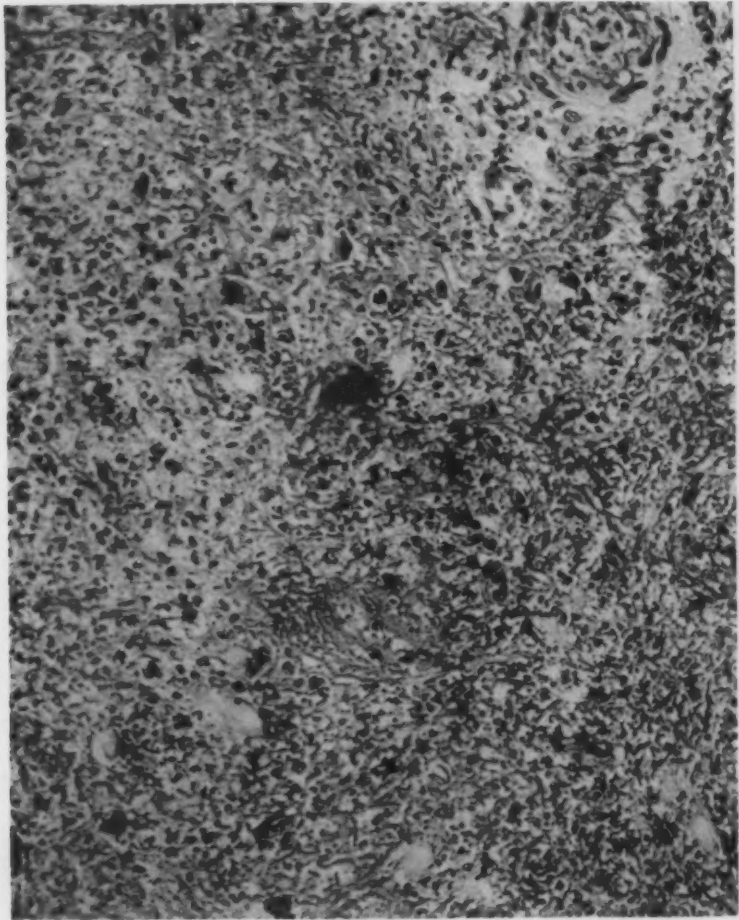


Fig. 4.—Biopsy specimen taken after ten weeks of local cold; approximately $\times 150$. Note the almost complete disappearance of tumor cells with a background of necrotic tissue, the nature of which cannot be identified.

on simultaneously with the phenomena of regression observed in respect to the tumor cells.

The phenomena of regression may be briefly summarized as follows: Granular, hydropic and fatty degenerative changes may be observed in the cytoplasm of the cells, depending largely on their individual char-

acter and origin. In many instances the ratio of nucleus to cytoplasm is so distorted that little evidence of cytoplasmic change can be observed. These cytoplasmic changes are followed by or associated with nuclear degeneration. More frequently pyknotic condensation of the nucleus is observed, but on occasion karyolytic swelling of the nucleus occurs, ending in actual rupture of the nuclear membrane. It has been our experience, as has already been indicated, that the more undifferentiated such tumor cells are, especially if they belong to the epithelial series, the more rapid and extensive are the regressive phenomena. Histologically, the effects of this physical agent, cold, are not so unlike the effects of that physical agent which has been more carefully studied in respect to cell changes, roentgen radiation. These merely represent physical agents occurring at different points on the physical spectrum, and the hypothesis is advanced again that with the withdrawal of the heat stimulus of cell growth through the application of cold, tumor tissue may well be rendered more susceptible to the harmful wavelengths of the roentgen ray, radium or other as yet undiscovered rays.

CHANGES IN DISTANT METASTATIC TUMOR UNDER REDUCED BODY TEMPERATURE

In this paper it is impossible to go into elaborate detail regarding the cell changes which have been observed in the numerous metastatic lesions in patients subjected to reductions of body temperature to levels ranging from 74 to 90 F. We can only generalize and state that, so far as we have been able to determine, the microscopic changes which are observed under these circumstances are similar in kind, varying only in extent, to those observed in primary tumor tissue submitted to local cold at temperatures ranging from 40 to 50 F. Individually, it is impossible to say that all such metastatic foci are uniformly affected. Indeed, at this barely subcritical level of temperature in many cases there seems to be no particular change in the progress of the neoplastic process. These differences are best observed perhaps in those few cases in which serial roentgenologic studies have been made on skeletal metastases. It has already been stated that in some instances regression can be observed; in other instances the process seems to be arrested, but no actual filling-in of such skeletal lesions is seen, and in others the metastatic process seems to progress uninterruptedly to its fatal outcome. One is undoubtedly somewhat influenced by the clinical response of these patients to the application of the low temperatures. The relief of pain, which is so striking, and the measurable reduction in the size of the lesions both locally and in regional lymph nodes containing metastatic foci make it almost impossible not to be somewhat prejudiced at the outset. We have attempted to avoid this factor so far as possible by

having a number of pathologists express their opinions regarding the individual slides without any accurate information in respect to the interval of time at which the specimens were obtained. It is only in this way that we have gradually come to the conclusion that, speaking generally, regressive changes may be observed with a fair degree of regularity in the majority of the tissues studied. That these changes are much less marked than those observed in the tissues from patients treated locally is apparent. That no appreciable change occurs is probably true in a small proportion of the cases. It is our impression that this absence of demonstrable degeneration is regularly true until after at least ninety-six to one hundred and twenty hours of general reduction of temperature. In some instances changes may not be found until a second period of ninety-six to one hundred and twenty hours has passed. From such studies, however, it is our feeling that if, with repeated applications of temperatures in the eighties, sufficient time elapses, such regressive changes can be found regularly.

SUMMARY

In this paper a brief summary of the pathologic studies in a series of some 60 cases in which patients with cancer were subjected to local or general reductions of temperature and in which autopsies were made is presented. The studies are divided into three major groups: first, those relating to the normal body tissues; second, those relating to tumor tissue subjected locally to temperatures of 40 to 50 F. for varying periods of time, and, third, those relating to metastatic tumor tissue in patients whose general temperature had been reduced to subcritical levels between 74 and 90 F.

Relating to the first group of non-neoplastic, or "normal," tissues, it may be said "a priori" that significant changes are the exception rather than the rule. In the series of 60 autopsies marked myocardial degeneration was found in 5 cases. In 3 of these it was associated with definite sclerosis of coronary arteries. In 3 cases acute pancreatitis was seen. All 3 of the patients had been accustomed to take relatively large amounts of morphine. In 4 cases a significant and persistent fall in blood pressure was observed. In 3 of these there was extensive bilateral adrenal metastasis. Bronchopneumonia other than the terminal event commonly encountered in cases of advanced malignant disease appeared chiefly in the postrefrigeration period, in association with metastatic involvement of the lungs. Its occurrence with and relationship to refrigeration, accordingly, is particularly difficult to evaluate.

With respect to the second group, temperatures of 40 to 50 F. applied locally to tumors regularly produced regressive changes going on to actual necrosis, even to the point of histologic clearance of the tissue of tumor cells in occasional instances.

In regard to the third group, the changes which were observed in metastatic tumor tissue from patients who were submitted to general reductions of temperature to 74 to 90 F. were similar in kind but varied greatly in degree in comparison with those resulting from application of the lower temperatures locally. In no case were regressive changes encountered until ninety-six to one hundred and twenty hours of refrigeration had been given, and in some cases no significant change seemed to occur even after three hundred hours of such low temperatures.

RADIATION PNEUMONITIS

EXPERIMENTAL AND PATHOLOGIC OBSERVATIONS

SHIELDS WARREN, M.D.

AND

OLIVE GATES, M.D.

BOSTON

With the increasing use of roentgen rays and radium for therapeutic purposes, the pathologic effects of radiation on normal tissues have steadily assumed more importance. When irradiation of tissues was resorted to chiefly as a palliative measure, the pathologic alterations of normal tissues due to irradiation were not so vital as they are today, when doses of 5,000, 8,000 and even 10,000 roentgens (r) are commonly used. Now, with the span of life frequently lengthened months to years, the effect of radiation on normal tissues takes on new significance.

Over thirty years ago it was pointed out by Wolbach¹ that after normal skin and subcutaneous tissues had suffered a sufficient degree of injury from radiation, complete repair did not take place and that degenerative and reparative processes might continue for some time, even years, after the last irradiation. This early study was an analysis of the nature of the damage to tissues subjected to roentgen rays and of their response. It has remained the authoritative account of the cutaneous changes. The effects on deep tissues are more difficult to determine as contrasted with those on the skin, and probably for this reason they have been given relatively little consideration. We propose in this paper to trace the early effects of different forms of irradiation of lungs of normal animals of different species, correlating them with observations on irradiated human lungs. For this purpose we have used roentgen rays generated at several voltages, radon and radioactive phosphorus.

During the past fifteen years there has been increasing interest in pulmonary damage from radiation, and the clinical signs and symptoms as well as the roentgenographic pictures resulting from such damage

From the Department of Pathology, Harvard Medical School, and the Laboratories of Pathology of the Huntington Memorial Hospital and New England Deaconess Hospital.

Dr. Joseph H. Marks contributed advice and facilities for irradiation; Dr. Richard Dresser permitted the use of the million volt x-ray apparatus of the Huntington Hospital, and Prof. Kenneth T. Bainbridge gave us a supply of radioactive phosphorus.

1. Wolbach, S. B.: J. M. Research **16**:415, 1909.

have been adequately reported. Thus far, postmortem observations have been rare, probably because it is seldom that a patient dies at the height of a reaction to radiation, when attention is focused on this condition. There are many difficulties in the interpretation post mortem of organic changes resulting from the use of radiation, dependent on the fact that the observer can see only one stage of an ever changing process in which several diverse factors may be or have been involved. Our method must be a piecing together of different kinds of evidence, roentgenologic, clinical, postmortem, experimental.

A study has been made by Warren and Spencer² of postmortem material from a series of 234 patients who had received some form of radiation, however slight, directed to the thorax. In this study it was found possible to recognize with accuracy a distinct disease picture as characteristic of certain phases of radiation reaction of the lung. But it was obvious that the process could not be followed through every stage. The fact that the material available for study was random—i. e., not taken with a view to studying changes from irradiation of the tissues—and often showed massive metastases as well as pneumonia militated against the study.

Many workers have attempted to duplicate the condition in animals to determine the nature of the tissue changes which produced the pulmonary reactions often transient but sometimes permanent seen in human patients following the use of radiation. These have been less illuminating than might be supposed, chiefly because so little was known of the changes in human lungs following irradiation that attempted extrapolation of experimental results was misleading. The experimental work varies greatly in significance and cannot be properly summarized, so divergent are the controlling conditions of experiments and the conclusions of the observers. Comprehensive reviews have come from Desjardins³ and Engelstad,⁴ who has himself made contributions in this field. The early work was focused chiefly on the effect on pulmonary tuberculosis. But recently there has been keen interest in the effect of radiation on normal lungs. Certain features have been consistently reported, such as fibrosis of the pleura with adhesions, and fibrosis of the lung parenchyma. Pneumonia, diffuse or the lobular type, and abscesses have been frequently described, as well as congestion, edema and atelectasis. These descriptions are almost monotonous in their uniformity, which contrasts strikingly with the great variation in the species used, the conditions of irradiation and the duration of the life of the animal afterward. Several careful workers have found no change

2. Warren, S., and Spencer, J.: *Am. J. Roentgenol.* **43**:682, 1940.

3. Desjardins, A. U.: *Am. J. Roentgenol.* **28**:271 and 421, 1932.

4. Engelstad, R. B.: *Acta radiol.*, 1934, supp. 19, p. 1.

in the lung following irradiation.⁵ From these observations, not always consistent, certain facts seem clear: that there is some variation in the sensitivity of different species, that some animals are less sensitive to radiation than man and that there is a dosage threshold below which no reaction is visible. In most reports it was indicated that no reaction occurred unless erythema had been produced. Probably the most complete and carefully controlled studies thus far published are those of Engelstad.⁴ He correlated his observations on the rabbit with careful variation of the physical factors, such as fractionation, the quality of rays and the relation of skin dosage to lung dosage.

Data on Experimental Production of Radiation Pneumonitis

Animal Group	Type of Radiation	Amount of Radiation		Time Since Last Radiation	
		Maximum	Minimum	Maximum	Minimum
51 rats	200 kilovolt roentgen rays	3,000 r	1,200 r	12 weeks	0
36 rats	100 kilovolt roentgen rays	2,100 r	1,600 r	8 weeks	1 day
11 rats	1,000 kilovolt roentgen rays	2,500 r	2,500 r	4 days	1 hr.
7 rats	Radioactive phosphorus	150 microcuries	30 microcuries	1 week	2 hr.
7 rabbits	200 kilovolt roentgen rays	3,600 r	300 r	5 mo.	0
2 rabbits	100 kilovolt roentgen rays	5,400 r	2,000 r	5 mo.	2 hr.
4 rabbits	Radon	2,800 millicurie hours	1,925 millicurie hours	6 weeks	5 weeks
2 dogs	200 kilovolt roentgen rays	4,800 r	1,200 r	1 week	3 days
1 pig	200 kilovolt roentgen rays	12,300 r	12,300 r	1 week

If the changes following irradiation of the lung are studied from the beginning, much of the present information can be ordered and clarified, giving a complete picture. In our experimental work we have, therefore, concentrated on early changes and have been more concerned with the production of a radiation effect than with contrasting methods of delivering radiation. With the smaller animals, rat and rabbit, we have used graded, relatively small amounts, with the intent to produce minimal changes and thus avoid introduction of such factors as hemorrhage or overwhelming inflammation, which may occur with necrotizing doses. With dogs, pigs and some rabbits, doses were used which we felt should cause definite, though not excessive, acute reaction. Animals were killed at intervals after irradiation. These data are appended (table).

5. Warren, S. L., and Whipple, G. H.: J. Exper. Med. **35**:187, 1922.

Before describing in detail the histologic changes seen in these experimental animals, it may be profitable to review those in human tissues.

The tissue reaction to radiation varies in intensity, in extent and somewhat in character. These variations hinge on the amount of radiation absorbed by the tissues, the rate at which it was given, the extent of fractionation, the character of the tissues, the amount of tissue damaged and the interval following irradiation. No variation in histologic or cytologic effects specific for wavelength has been noted with therapeutic rays.⁶

The picture of radiation effects in any tissue includes, first, changes in the cells irradiated, ranging from necrosis to various degrees of necrobiosis, distortion, vacuolation, gigantism and variation in secretory activity not only in cells directly exposed to radiation but in descendants of these cells; at times abnormalities of mitosis; sometimes calcification; second, changes in intercellular substance, varying from slight edema to deposition of fibrin, hyalinization or even necrosis of collagen and degeneration of elastica. From combinations of these fairly simple changes, the manifold tissue responses to radiation are built.

The histologic criteria for radiation pneumonitis used by Warren and Spencer² are as follows: swelling and distortion of the alveolar lining cells with some desquamation; formation of a hyaline membrane, usually closely adherent to the alveolar wall, and, in conjunction with these, edema, congestion, fibrosis of alveolar walls of a dense hyaline type and swelling of arterial walls. Inflammatory cell reaction of any type was discounted, as it was impossible to differentiate cell infiltration as a response to irradiation from that present as a response to infection. The present study confirms, clarifies and extends these criteria.

ALVEOLAR AND ATRIAL CELLS

The cells lining alveoli and atria may show changes as a result of a sufficient degree of irradiation which in certain instances are distinctive and are essentially similar regardless of species. While not always present, they are quite readily apparent in man, dogs, pigs and rabbits. The factors determining the degree of alteration of these cells have not yet been evaluated.

The earliest change is an increase in cell size, with or without nuclear hypertrophy. The cells usually maintain the normal relation to the wall, thus producing a fairly uniform lining of large alveolar cells. This is identical with the picture so often seen in chronic inflammation. From this step there is progression to many and varied forms, the cells becoming often extremely hypertrophic and bizarre and not unlike the giant cells seen in some tumors. The latter forms we have never seen in other than irradiated lungs. As the cells take on these extraordinary shapes they tend to separate from the basement membrane. At times

6. Warren, S., and Fogg, L. C.: *Proc. Soc. Exper. Biol. & Med.* **30**:91, 1938.

tenuous cytoplasmic processes maintain the continuity of the distorted cells with one another or with the alveolar wall. The cell size generally increases with the degree of distortion of outline, sometimes to ten or more times the original size. Often there is no change in the texture or the staining property of the cytoplasm, even though the cell as a whole is markedly distorted. There is often a slight tendency toward basophilic staining; the texture is homogeneous, without evidence of necrobiosis, and the cell boundary is usually clearly defined, though often without a distinct cell membrane. There is no evidence of phagocytic activity. Vacuolation is rarely noted.

As the cell increases in size, the nucleus becomes larger and rather more vesicular, with marked clumping of the chromatin and the presence of one or more large nucleoli. These nucleoli may rival those of regenerating hepatic cells or

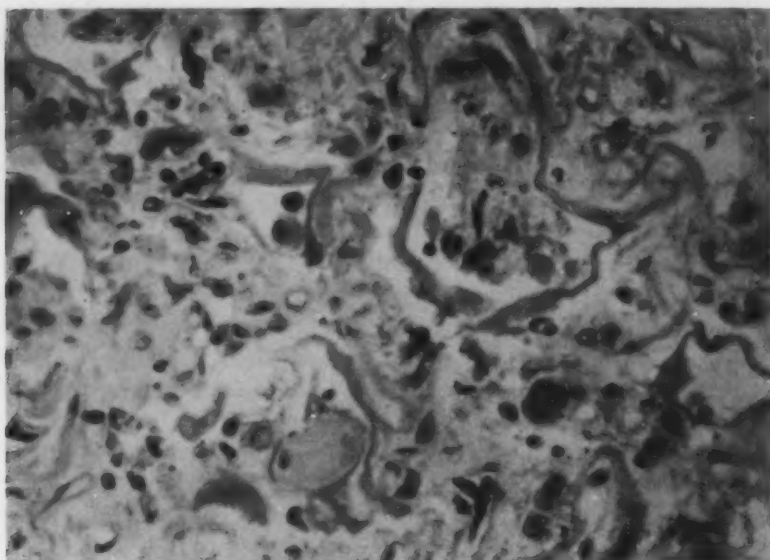


Fig. 1.—Abnormal alveolar lining cells and early hyaline membrane in human lung that had been irradiated with 200 kilovolt roentgen rays; $\times 266$.

of the cells of certain types of carcinoma. As a rule, the nucleus remains single and maintains an ovoid contour, but some cells have several nuclei and others strangely irregular ones. Mitotic figures are not seen in these large cells, although normal alveolar cells in animals and occasionally those in man may be seen in mitosis.

In some instances all the cells lining an alveolus may be greatly hypertrophied and form an eosinophilic syncytial band with unevenly spaced nuclei. In a closely adjacent alveolus only a single cell may be hypertrophied. There is no uniformity of change from one alveolus to another, although if cell hypertrophy and distortion are present some degree of change is seen in all alveoli in a given field. While the hypertrophied, anaplastic cells may or may not be attached to the wall, they rarely are free in the center of the lumen, separation if any being slight.

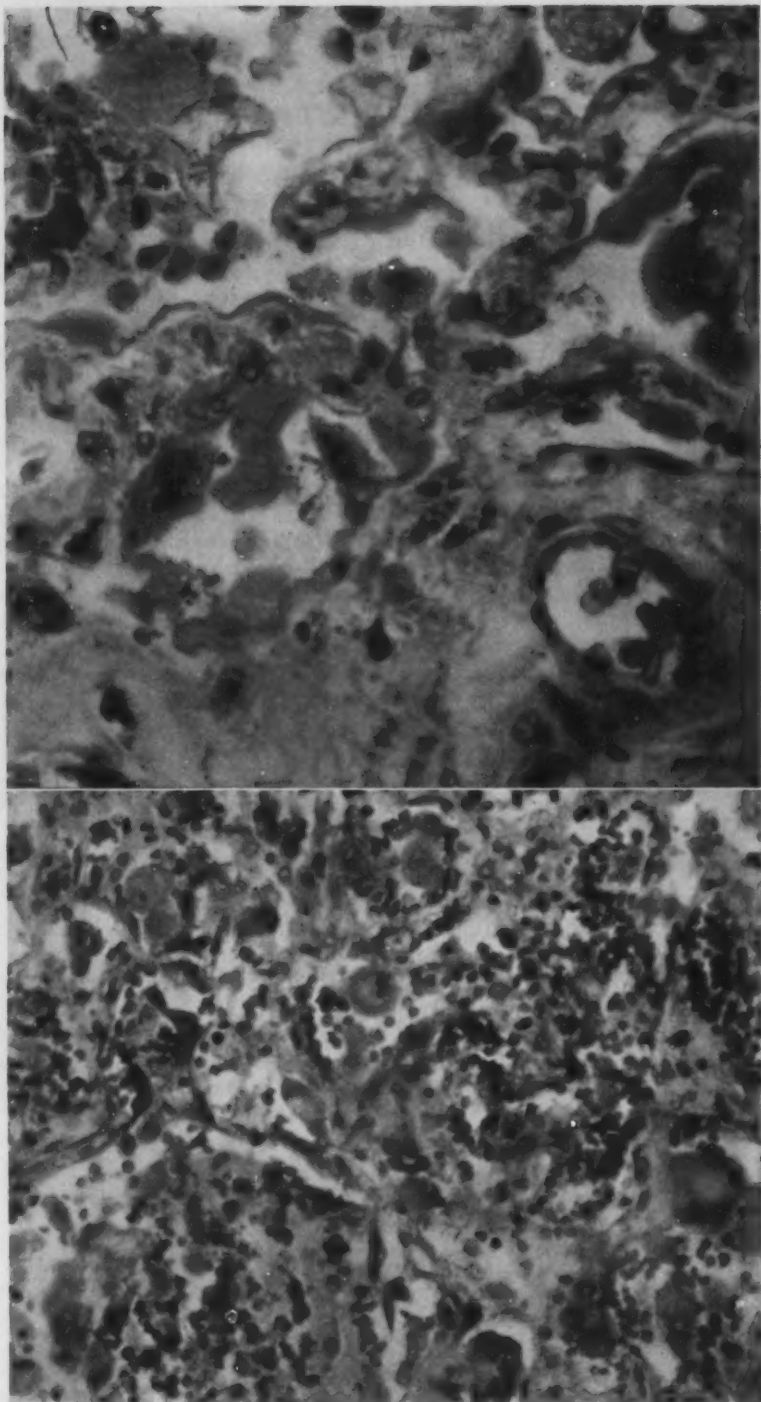


Fig. 2.—Upper part: Abnormal alveolar lining cells and early hyaline membrane in human lung (same lung as in figure 1) that had been irradiated with 200 kilovolt roentgen rays; $\times 600$. Note the continuity of alveolar cells and membrane and the fusion of membrane and wall. Note also the hyaline thickening of the alveolar walls.

Lower part: Altered alveolar lining cells of a rat's lung; $\times 300$. The animal was killed immediately after three daily doses of 400 r each of 200 kilovolt roentgen rays.

The more marked changes are less often seen in animals than in man and are most striking in the dog, the more usual picture in the rat and the rabbit being moderate hypertrophy, with only rarely anaplasia.

This cell hypertrophy in its most marked degree is easily distinguished from the familiar hyperplasia seen in inflammatory conditions of long standing—for example, chronic pulmonary tuberculosis in man and experimental gas poisoning in dogs. The hyperplastic cells tend to be cuboid, rarely exceed the macrophage in size and may even grow in sheets simulating carcinoma. These sheets of cells are usually seen in atria near bronchioles. This is quite different from the hypertrophy and anaplasia of irradiated lungs, the nearest approach to which is that described in epidemic influenzal pneumonia⁷ and that following insufflation of hydrochloric acid.⁸

This alveolar cell change we have found to a certain extent in all lungs, human and animal, with definite radiation reaction, but it is not always commensurate with other phases of the reaction. In some instances these cells become necrotic and fuse with the hyaline membrane described in the next section.

We interpret the typical change of the alveolar lining cells as the result of injury to the cells, which respond primarily by hypertrophy and anaplasia and only to a minor degree by proliferation, in contrast to the hyperplastic reaction subsequent to chronic inflammation, in which proliferation predominates. This difference in reaction is probably related to the nature of the injury.

HYALINE MEMBRANE

Another characteristic, though not constant, reaction in the irradiated lung is the formation of a hyaline membrane. The term "hyaline" is used here as a purely descriptive term indicating the homogeneous glassy eosinophilic appearance rather than its chemical structure. The frequency of this reaction in human subjects following irradiation has been stressed in a previous publication.² It is seen infrequently in animals. Although striking in man, it is less important from the pathognomonic standpoint than the epithelial changes. A hyaline membrane is one of the most constant features of epidemic influenza,⁹ and has been described in a variety of pneumonic lesions,¹⁰ including those of plague,¹¹ pneumonia of the newborn,¹² aspiration of amniotic fluid¹³ and irritant gas poisoning.¹⁴ In a series of lungs from 1,000 consecutive autopsies which we have recently reviewed, extensive hyaline membrane was found in 5 cases,¹⁵ in all of which, we

7. Winternitz, M. C.; Wason, I. M., and McNamara, F. P.: *The Pathology of Influenza*, New Haven, Conn., Yale University Press, 1920.

8. Winternitz, M. C.; Smith, G. H., and McNamara, F. P.: *J. Exper. Med.* **32**:205, 1920.

9. Wolbach, S. B.: (a) *Bull. Johns Hopkins Hosp.* **30**:104, 1919; (b) *Arch. Int. Med.* **32**:517, 1923.

10. Brannon, D., and Goodpasture, E. W.: *Arch. Int. Med.* **34**:739, 1924.

11. Strong, R. P.; Crowell, B. C., and Teague, O.: *Philippine J. Sc.* **7**:203, 1912.

12. Johnson, W. C.: *Proc. New York Path. Soc.* **23**:138, 1923.

13. Farber, S., and Sweet, L. K.: *Am. J. Dis. Child.* **42**:1372, 1931.

14. Winternitz, M. C.: *Pathology of War Gas Poisoning*, New Haven, Conn., Yale University Press, 1920.

15. These were the only cases in the series in which there was radiation to the chest.

learned later, there had been considerable radiation to the chest. In 4 other cases of the series, hyaline membrane was found in one or two isolated alveoli. The membrane in the latter cases, in which there had not been irradiation of pulmonary tissue, was like that in irradiated lungs except that it was slight in amount and spotty in distribution. In a well marked radiation reaction this membrane is seen in all the alveoli of several fields.

The membrane lies against the alveolar wall, at times close to it but at other times slightly separated, yet rarely lying free in the lumen. The alveoli lined by the hyaline membrane are nearly always empty, distended, usually to a marked degree, and in striking contrast to adjacent partially collapsed alveoli, which may or may not be filled with exudate. Its close approximation to the wall and at times fusion with it, a part of the alveolar wall being converted into a homogeneous band of similar hyaline appearance, suggest that the relation may be closer than mere juxtaposition. Occasionally fibroblasts extend from the wall into the hyalin, but there is never any organization. Necrotic alveolar cells often merge with the membrane. It is characteristically broad rather than threadlike. The broken ends of the membrane may have a finely fibrillar structure, which merges into the otherwise glassy membrane. It is not easily confused with fibrin undergoing hyaline change, such as one frequently sees lying free in the alveolar lumen in pneumonia. Although it does not stain as fibrin with Mallory's aniline blue, phosphotungstic acid-hematoxylin, or other stains, this does not signify that partly autolyzed fibrin may not be one of the constituents. Occasionally a few macrophages may be incorporated in the membrane at its periphery. Vacuoles are rarely seen in it, but with sudan IV or osmic acid fine droplets giving a fat reaction are commonly seen throughout its structure.

The membrane is easily differentiated from the granular albuminous precipitate which often condenses at the periphery of an alveolus in pulmonary edema. Although many of the lungs studied showed various stages and types of pneumonia, with and without organization, the membrane was never seen in fields associated with any considerable cellular exudate.

Wolbach's descriptions of the hyaline membrane in epidemic influenza in 1919^{9a} and 1923^{9b} emphasized the association with emphysema. He further pointed out the causal relationship between forcibly inspired air and the formation of the membrane, showing conclusively that air under pressure may change the appearance of an exudate whether in the lung or in supporting tissues, as the mediastinum.

Farber and Wilson¹⁶ drew similar conclusions from the results of a series of ingenious experiments designed to test the various factors which might be of importance in the formation of the hyaline membrane. The presence of an exudate old enough to be partly autolyzed and exaggerated respiration were felt to be the two essential conditions and probably the chief ones concerned in its production in disease. Injury to alveolar walls was considered unimportant.

In nearly all of our cases with hyaline membrane some exudation is shown, chiefly fluid, most marked in parts of the lung without the membrane. Atelectasis and emphysema in closely adjacent fields are also characteristic, the alveoli lined with the membrane being much larger than others close by.

The striking epithelial change which is seen in our irradiated animal and human lungs and the frequently close approximation of the membrane to the alveolar wall, which itself may show a partial transformation into hyaline membrane, incline us to believe that injury to alveolar walls and lining epithelium may play a part in its formation.

16. Farber, S., and Wilson, J. L.: Arch. Path. 14:437 and 450, 1932.

ALVEOLAR WALLS

In animals, edema of alveolar walls is seen early but is often irregularly distributed. Congestion, also early and often marked, is usually uniform throughout the irradiated part. Focal hemorrhages into the alveoli may be associated with this congestion. Another early change, associated with edema and congestion, is marked cellularity of the alveolar walls due to swelling of so-called septal cells and

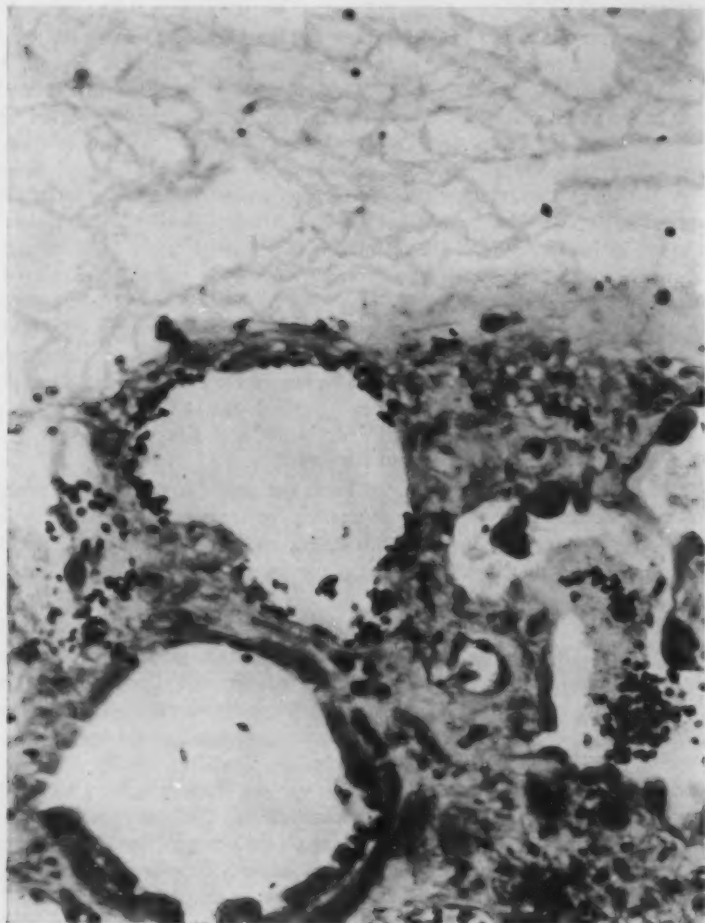


Fig. 3.—Abnormality of alveolar lining cells and partial atrophy and metaplasia of bronchial epithelium in the lung of a pig given 200 kilovolt roentgen rays to a total of 12,300 r in divided doses, and killed one week after the last irradiation; $\times 256$. Note the extreme edema of the lobular septum.

in part to swollen endothelial cells. Wandering mononuclear cells are seen in small numbers, chiefly lymphocytes or plasma cells.

Thickening and splitting of the elastic fibers occur somewhat later than edema, congestion and nuclear swelling but are present when there is a well established reaction. Since our interest has been primarily in the early reaction to radiation,

the experiments were not designed to show the late stage of hyalinization and fibrosis, descriptions of which fill the literature. Degeneration of collagen of alveolar ducts is not seen in most animals but is distinct in the dog and the pig. This process may be responsible for some of the hyalinization of the walls of alveolar ducts and alveoli in man. Partial, irregular atelectasis and emphysema are nearly always present.

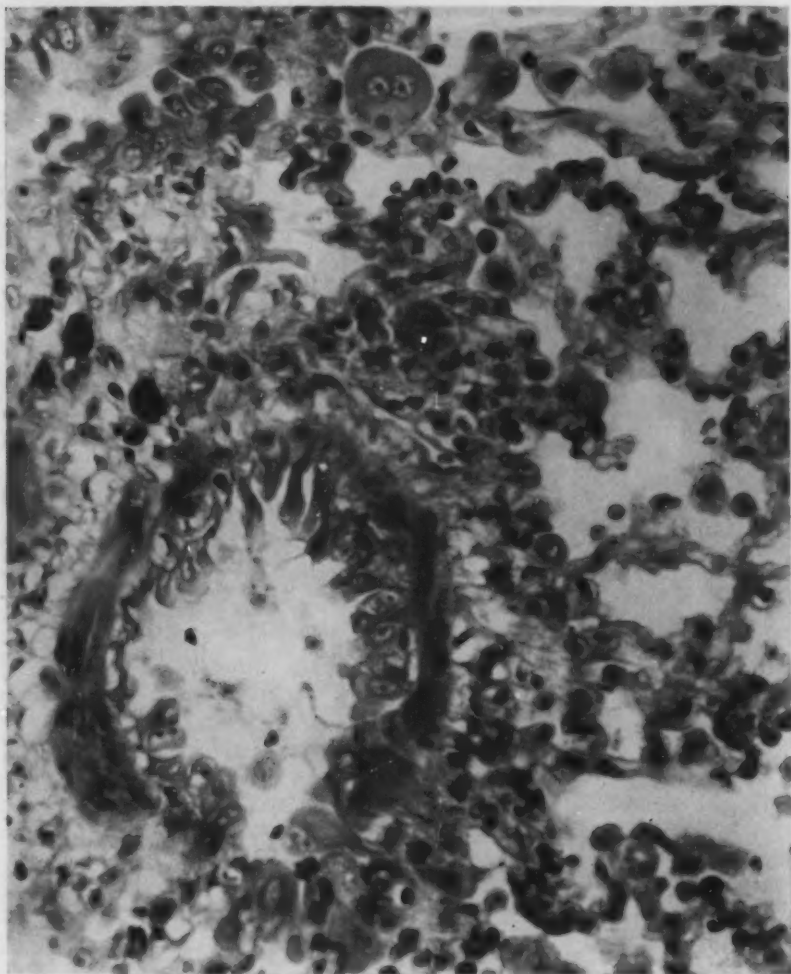


Fig. 4.—Abnormality of alveolar lining cells and of bronchial epithelium in the lung of a dog given 160 kilovolt roentgen rays to a total of 4,800 r, in divided doses, and killed three days after the last irradiation; $\times 400$.

When congestion and edema alone have been seen in human lungs, it has not been possible to attribute them with certainty to radiation, although they are present in lungs with epithelial hypertrophy and hyaline membrane. We have observed dense hyaline fibrosis in human irradiated lungs, often accompanied by fragmenta-

tion of elastica. These changes are important in relation to the occurrence of focal emphysema. We feel that this hyaline fibrosis may be the last phase of radiation reaction, but it cannot always be differentiated with certainty from the fibrosed hyaline alveolar walls frequently seen in old age.

BRONCHI AND BRONCHIOLES

Bronchial epithelium, in contrast to that of alveoli, is relatively stable. In some of the animals with other evidences of radiation reaction there is excessive mucus secretion, with partial desquamation of epithelium. The increased number of mucus cells may represent a degenerative change of the ciliated cells,¹⁷ which are decreased in number. Columnar cells become cuboid, usually with loss of cilia, and

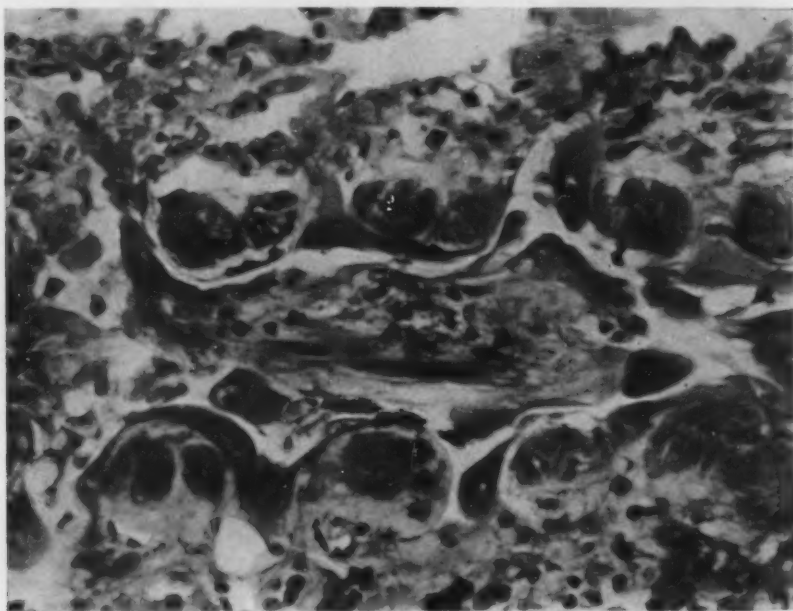


Fig. 5.—Bronchiolar change with marked epithelial abnormalities in the lung of a dog given 160 kilovolt roentgen rays to a total of 4,800 r, in divided doses, and killed three days after the last irradiation; $\times 400$. Note the retained secretion and exudate.

occasionally reduplicated, forming two and rarely three layers, but the true metaplasia to keratinized stratified epithelium of vitamin A deficiency is never reached.¹⁸

In the human bronchus somewhat minor changes have been observed which could not by themselves be ascribed definitely to irradiation. Nevertheless their resemblance to the radiation reaction of animal bronchial epithelium makes it more

17. Miller, W. S.: *The Lung*, Springfield, Ill., Charles C. Thomas, Publisher, 1937.

18. Wolbach, S. B., and Howe, P. R.: *J. Exper. Med.* **42**:753, 1925; **57**:511, 1933.

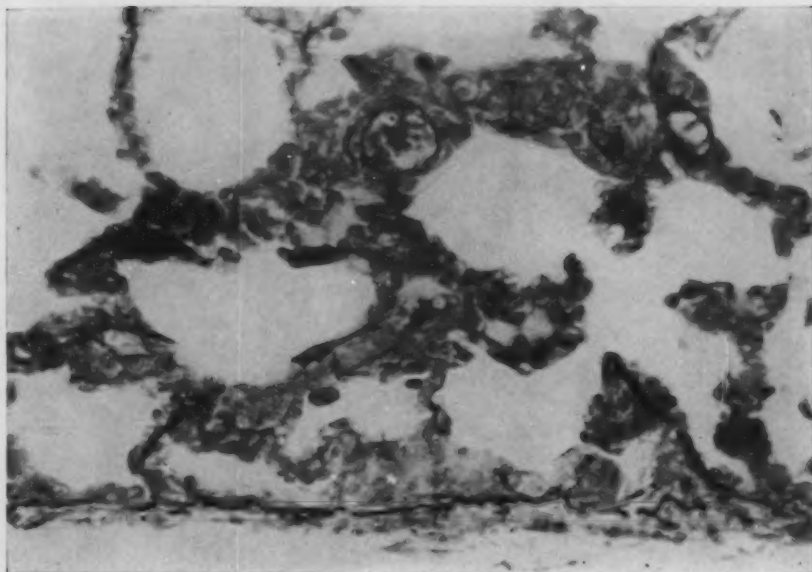


Fig. 6.—Weigert elastic tissue stain of a portion of an unirradiated lobe of a pig's lung (same animal as in figure 7); $\times 256$.

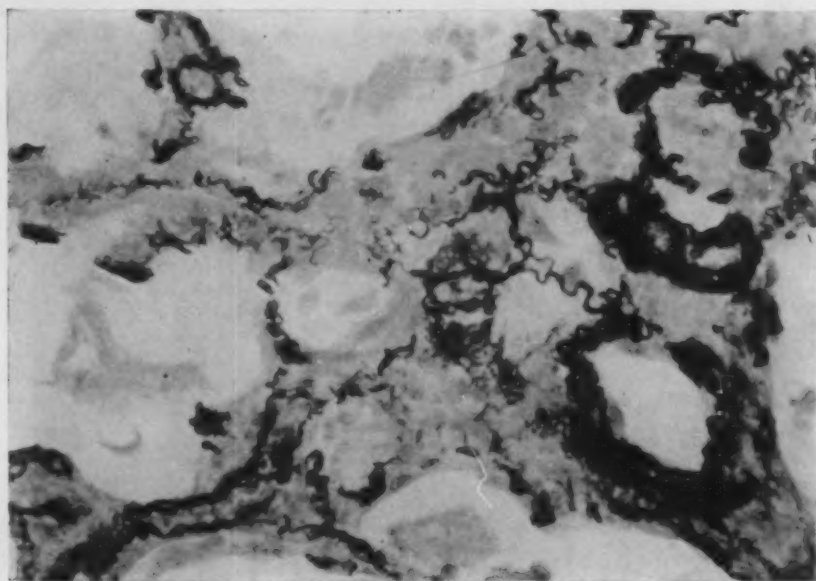


Fig. 7.—Weigert elastic tissue stain of a portion of a lobe of a pig's lung which had received 12,300 r over four and one-half months; $\times 256$.

than likely that they are the result of radiation. The partial loss of cilia and the increased secretion may be the cause of the troublesome irritative cough of radiation pneumonitis, a mechanism stressed by Mallory¹⁹ in pertussis.

Bronchiolar epithelium more nearly approaches the responsiveness of the alveolar lining cells. In the dog and pig there is anaplasia comparable to that of the alveolar epithelium present to some extent in all species. These anaplastic bronchiolar epithelial cells may be very large, multinucleated and often syncytial. Anaplasia and stratification of lesser degree, similar to that occurring in the bronchi, are seen in the bronchioles as well. Anaplastic changes are not seen in human subjects.

No very clearcut change of the supporting connective tissue or of muscle is seen. In 2 rabbits receiving 4,800 r there was slight fibrosis of the submucosa. Some swelling of smooth muscle was observed in these 2 rabbits and in dogs and

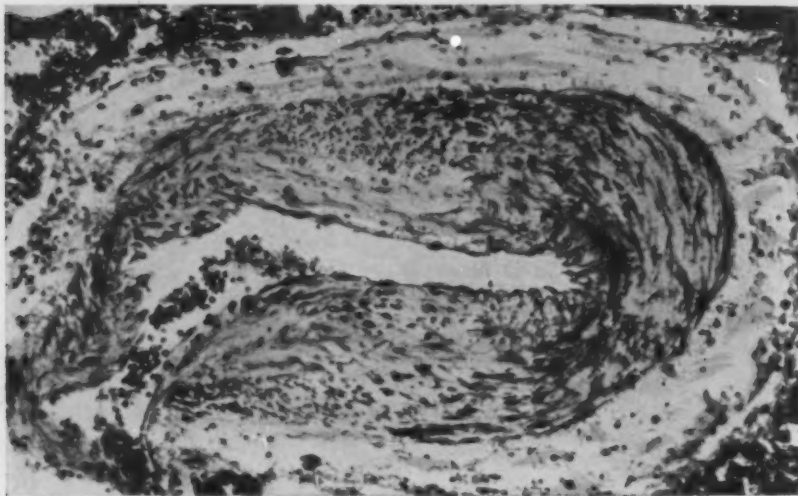


Fig. 8.—Edema of arteriole in a rabbit's lung; $\times 128$. The animal died during a second dose of 200 kilovolt roentgen rays, of 400 r, the doses being two days apart.

pigs. The cartilage has been normal in the irradiated lungs of animals and human beings we have seen.

No change is present in the lymphoid nodules in our animals except in 2 rabbits which received a total of 4,800 r and were killed five days after the last dose, 600 r. In these animals the lymph nodules had almost entirely disappeared. The rarity of lymphoid changes is probably explained by the facts that relatively light doses were used and death occurred at a sufficient interval afterward to permit regeneration.

LYMPHATICS AND BLOOD VESSELS

In animals and in some human patients varying degrees of dilatation of peribronchial and perivascular lymphatics occur, usually commensurate with the edema. This may be extremely marked.

19. Mallory, F. B.: *Principles of Pathologic Histology*, Philadelphia, W. B. Saunders Company, 1914.

With the exception of larger blood vessels, distinction between pulmonary arteries and veins, once radiation changes have appeared, is extremely uncertain. In animals the changes in the blood vessels other than edema are not striking. Some of the medium-sized and large arteries show endothelial swelling and vacuolation. Edema is often marked in the entire wall of both vein and artery. Rarely cellular infiltration occurs, not definitely ascribable to the radiation. Coarsening and reduplication of the elastica is noted only with higher dosages and is then often quite marked. The change in the human elastica is striking in some cases, and in these there is often observed as well marked hyaline swelling of both veins and arteries, but senescence cannot always be ruled out as the causative agent.

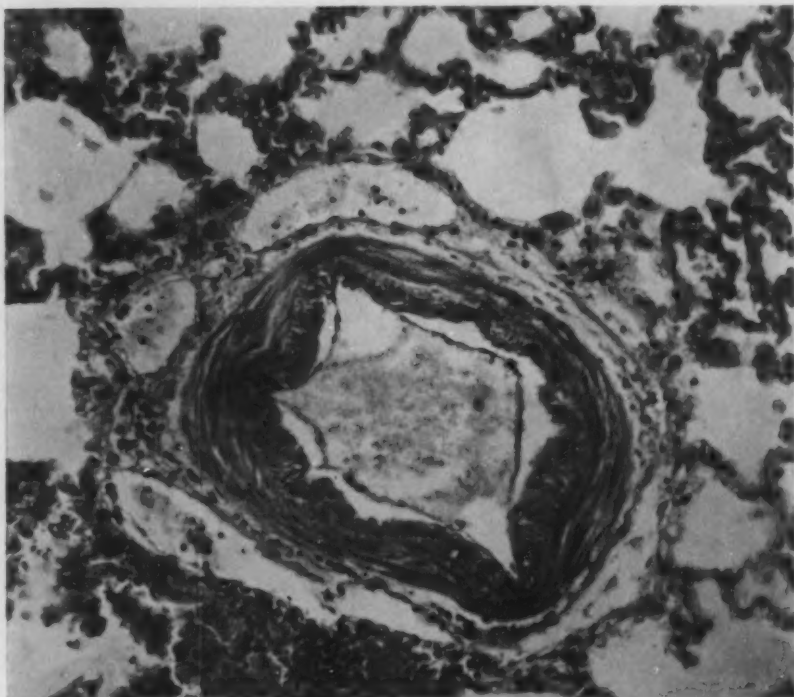


Fig. 9.—Mild edema and periarterial lymphectasia in a rabbit's lung; $\times 200$. The animal was killed two weeks after the last of five daily doses of 200 kilovolt roentgen rays, of 400 r each.

It is rarely possible to demonstrate changes in the smooth muscle of the vessels, but in a few instances there appears to be some vacuolation and in others partial disappearance of smooth muscle cells. The alveolar capillaries show no changes beyond congestion, occasional ectasia and in some instances very slight endothelial swelling.

INTERLOBULAR SEPTUMS AND SUPPORTING CONNECTIVE TISSUE

The general pulmonary edema seen early in all animals causes a striking increase in the extent of the supporting fibrous tissue, septal, peribronchial and perivascular.

In figures 10 and 11 are shown irradiated and nonirradiated parts of the same porcine lung. We have never seen a similar degree of edema in human lungs which we could with confidence attribute to radiation. From the general similarity of

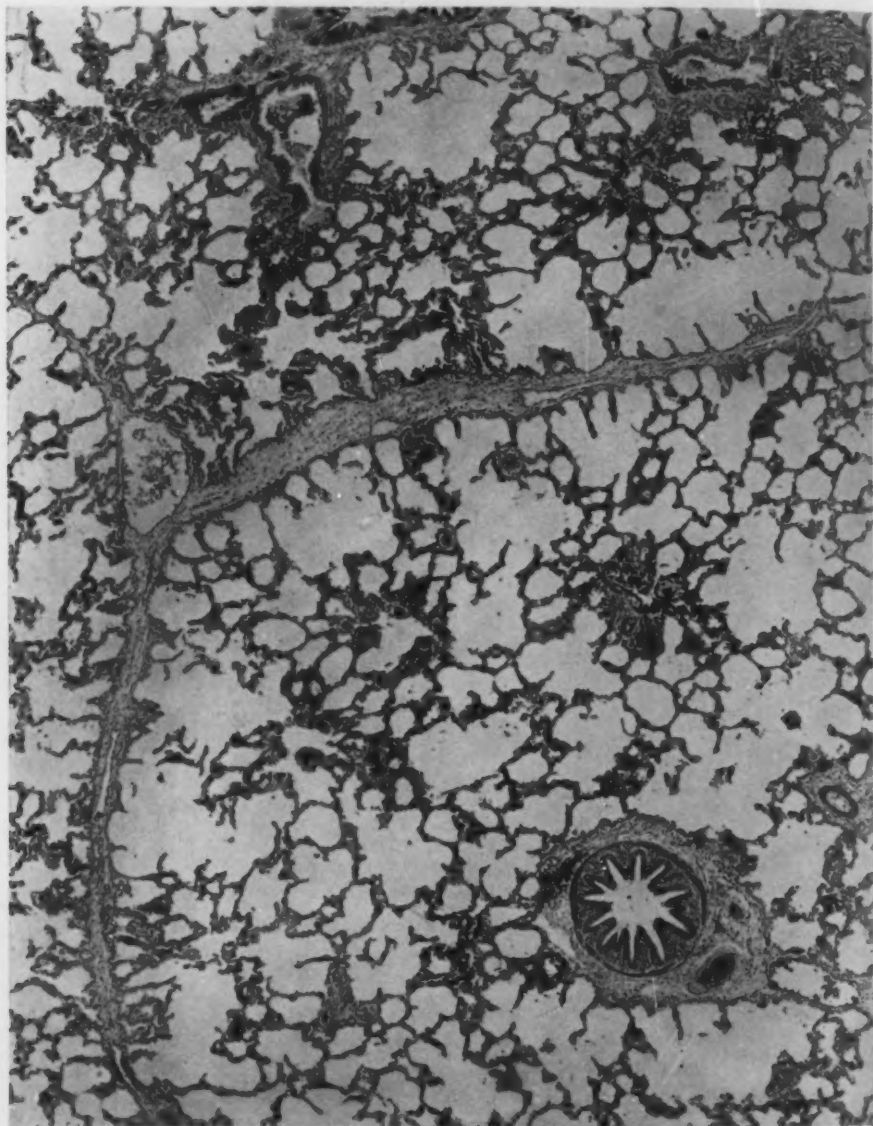


Fig. 10.—Normal lobe of a pig's lung to show the general architecture; $\times 36$.

radiation reaction in human and animal lungs it is fair to assume that a comparable edema may occur. This would account in part for the roentgenographic picture of transient radiation pneumonitis.

Focal hemorrhages into supporting fibrous tissue are occasionally seen.



Fig. 11.—Irradiated lobe of a pig's lung (same animal as shown in figure 10) to show edema and alveolar change; $\times 36$.

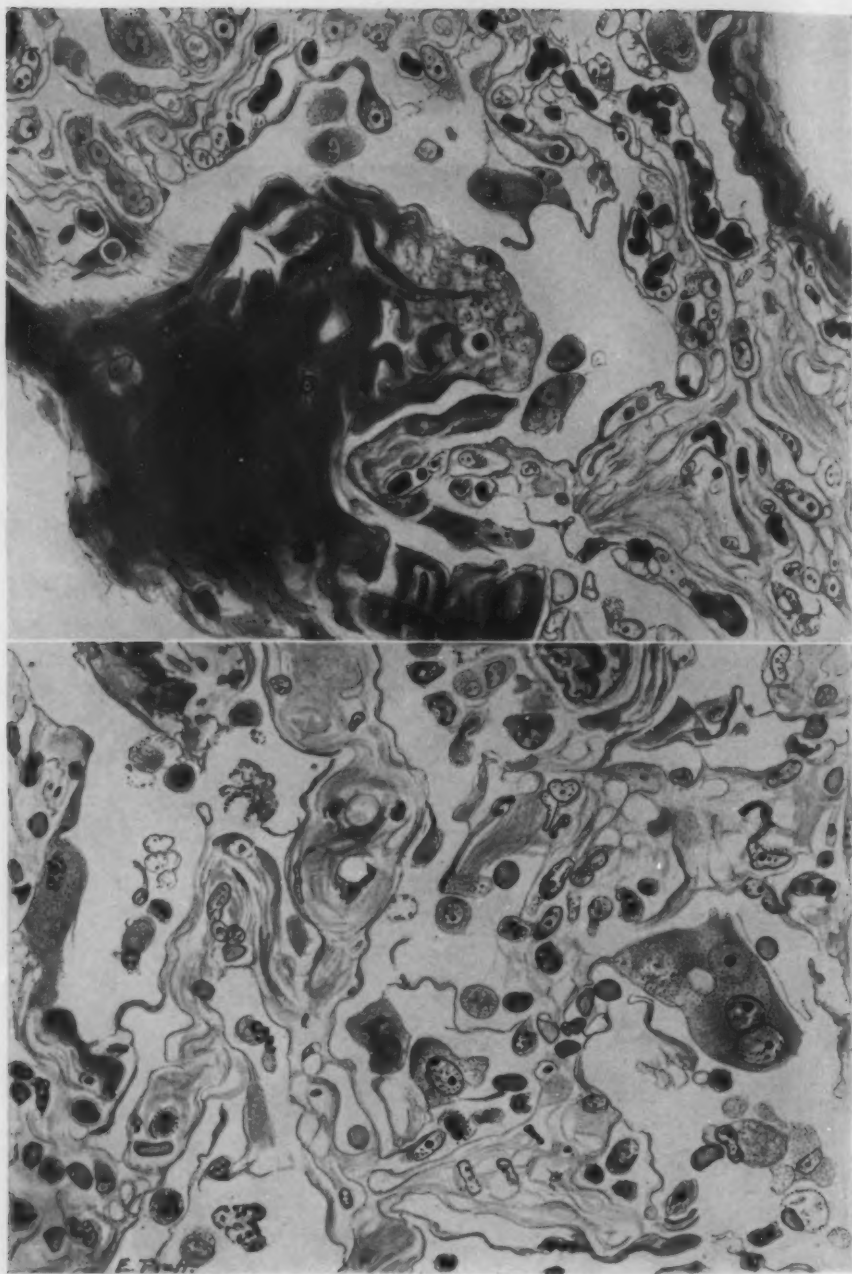


Fig. 12.—Upper part: Marked hyaline membrane in human lung—same lung as in figures 1 and 2 (upper part); $\times 600$. Note the extent to which such membrane may build up mass. In the upper right corner of this part is hyaline membrane partly fused with wall. Note abnormalities of the alveolar cells.

Lower part: Abnormality of alveolar lining cells and early hyaline change and thickening of alveolar walls in same human lung; $\times 600$.

No evidence of fibrosis is seen in our animal lungs. In human lungs fibrosis of the septums as part of general sclerosis is not infrequent, but here again there is no definite evidence that it is the result of irradiation rather than of inflammation.

PLEURA

On theoretic grounds one would expect the pleura and fibrous septums to be the last to react to irradiation other than by the development of edema, since these structures are composed of quite inert fibrous tissue. This is confirmed by our experiments. In a few animals there are slight to marked swelling and vacuolation of the mesothelial cells, sometimes accompanied by edema and rarely by slight hyalinization of the underlying connective tissue. The elastica shows degeneration resulting in swelling and fragmentation. Pleural effusions and fibrosis are not present.

Radiation pneumonitis of man is not clearly associated with pleural reaction other than edema and congestion. In case 19 of Warren and Spencer,² in which the typical "pleuro-pneumonitis" of the roentgenologist was seen on roentgenographic examination some months before death, the pleura in the field of irradiation was normal, adhesions being present only on the pleural diaphragmatic surfaces. There are many possible factors in man, such as metastases or concurrent infection (particularly in the patients with mediastinal or primary pulmonary tumor), which might reasonably be responsible for pleural adhesions.

COMMENT

The reaction of the human lung to radiation is a clinical entity of increasing importance. It is represented by definite pathologic changes, which are reproducible in animals with varying degrees of fidelity.

By experiments in four species we have been able to follow the progression of the response to irradiation up to a stage in the reaction which has been observed with considerable constancy in man. Congestion, edema, exudation, tissue injury (epithelial and mesenchymal) occur in different combinations, the emphasis shifting from one to another with varying conditions. This reaction is not constant either between species or individuals of a species. Certain stages of the reaction cannot be recognized by purely histologic evidence.

No one of these reactions alone is peculiar to radiation effects. But, as Wolbach¹ pointed out in the skin, so in the lung certain combinations of tissue response are so characteristic of the reaction to radiation as to be pathognomonic. Epithelial anaplasia, alveolar and bronchial, ruptured and reduplicated elastica and hyaline membrane lining alveoli, combined, we have seen in no other disease process. The first two of this triad are seen in irradiated skin. The third occurs rarely in a variety of inflammatory conditions in the lung and has been shown to result from acute alveolar emphysema with exudation which, it is fair to assume, has some unusual constituents, considering the rarity of the membrane as against the frequency of emphysema and exudation.

The constancy of edema and congestion combined with epithelial changes in irradiated animals makes it probable that the injury to the alveolar cells, and possibly to the capillary endothelium as well, results in an exudate rather different from that of ordinary pneumonia. The injury to the alveolar cells and walls is an important factor in the formation of the hyaline membrane.

The time sequences are clearly shown in a series of rats in which 30 to 159 microcuries of radioactive phosphorus was injected. Congestion and edema with focal hemorrhages but no cellular exudation appeared after twenty-four hours and during the next few days were overshadowed by proliferation and swelling of the alveolar lining cells, as well as by polymorphonuclear infiltration. Mitosis in alveolar cells is frequent on the fifth and sixth days after injection. Irregularity in the bronchial mucosa appears about the third day, and by the seventh day considerable anaplasia has gradually developed. These changes, induced by beta rays, will be reported later in greater detail.

In irradiated lungs degeneration of the elastica is probably important in producing emphysema. The cough and respiratory difficulty commonly present in human radiation pneumonitis may be due to increased bronchial secretion, impairment of ciliary function and to edema. These in turn may produce atelectasis, aggravate emphysema and lead to infection.

All of the early changes we have described are such as to produce distinct clouding of the lung fields on roentgenographic examination, and if not too severe they permit complete restoration to normal structure, as has been observed. It is not known whether the hyaline membrane is eventually autolyzed. We have never seen evidence of organization of the membrane.

Our observations confirm those recorded in the literature except for a few important differences. Though several authors have described changes in alveolar lining cells, so far as we can judge from descriptions and pictures the changes have been less prominent and apparently of a different nature from ours. Thus, Granzow²⁰ dismissed the epithelial changes with a single sentence: *Als Früh- und Spätfolge finden wir an den "Alveolarepithelien" viele Formen der regressiven Zellveränderung, vakuolige Degeneration, starke Ablösung und Ausfüllung der Alveolen mit den Zelltrümmern* (As early and late sequels, we find on the "alveolar epithelia" many forms of regressive cellular changes, vacuolar degeneration, considerable detachment and filling of the alveoli with the cellular debris). Ludin and Werthemann's²¹ figure 4 shows vacuolated cells, called epithelial but resembling macrophages rather than

20. Granzow, J.: Arch. f. Gynäk. **151**:612, 1932.

21. Ludin, M., and Werthemann, A.: Strahlentherapie **38**:684, 1930.

hyperplastic or anaplastic epithelial cells. On the other hand, there is almost unanimous agreement on the changes in the bronchial epithelium.

Hyaline membrane, characteristic of a phase of human radiation pneumonitis rarely seen in animals, we have not found described as a radiation effect.

We are in accord with most observers in regard to the importance of congestion, edema, petechial hemorrhage and lymphangiectasia as early and constant elements in the reaction. However, we have not seen the striking pigmentation, presumably hemosiderin, often described (Wohlauer;²² Engelstad;⁴ Pappenheim and Plesch²³).

The tendency in the literature has been to emphasize fibrosis, inflammation and pleural change,²⁴ overlooking the less striking and often obscured underlying changes. The absence of fibrosis in our animals is due in part to the doses and spacing of radiation. It may also be correlated with the absence of any considerable inflammation in our animals, a condition which nearly all authors describe as more or less extensive throughout irradiated human and animal lungs, especially those given heavy radiation in either single or repeated doses. It seems probable that severe injury of the tissues following the use of radiation increases their susceptibility to infection. The possible accentuation of already existing inflammatory changes by radiation may be a factor but is one which we are not at present prepared to discuss.

The relation of radiation reaction and pulmonary infection is an important but complicated one which is outside the scope of this paper. There is some clinical as well as experimental evidence which indicates that inflammation of the lung, as of the skin, may render the tissues more sensitive to radiation.

Extensive fibrous and pleural adhesions in general occur only as a result of actual pleural necrosis or inflammatory reaction. Many of the reports on experimental work stress fibrosis, both pulmonary and pleural, in conjunction with severe inflammation, necrosis and abscesses. Such pulmonic inflammatory lesions may well be secondary to bronchial epithelial change and loss of cilia due to radiation effect. The abscesses and fibrosis noted by Engelstad⁴ and others may be interpreted to support this origin for pleural changes.

Many of the experimental doses mentioned in the literature have been so massive as to be beyond comparison with those for human beings and to represent radiation effects on lungs already grossly pathologic because of the earlier irradiation.

22. Wohlauer, F.: *Deutsche med. Wchnschr.* **35**:1704, 1909.

23. Pappenheim, A., and Plesch, J.: *Ztschr. f. exper. Path. u. Therap.* **12**:95, 1913.

24. McIntosh, H. C., and Spitz, S.: *Am. J. Roentgenol.* **41**:605, 1939.

One fairly constant finding in the human patients is the displacement of the mediastinum toward the affected lung. This, we believe, is not so much the result of adhesions or "traction" as of atelectasis, and the same general mechanism is involved as in the mediastinal displacement in pneumonectomy.

In one other respect have our observations differed from those previously described, and this is the degeneration of the elastica of alveoli, pleura and vessels, occurring with any well established reaction. The only specific mention we have found in the literature is that by Ludin and Werthemann,²¹ who described diminution and complete disappearance of elastic fibers following irradiation.

Striking changes in blood vessels have not been described. Pappenheim and Plesch²² described endothelial injury after intravenous and oral administration of thorium x. In our animals we have seen some edema of the vessel wall with possible degeneration of the muscularis in some instances, rarely leukocytic infiltration and occasionally endothelial swelling.

The absence of any appreciable change in the lymphocytes in the majority of our animals is undoubtedly due to the lighter dosage of radiation. Engelstad⁴ showed that, while degeneration of lymphocytes in the follicles starts almost immediately following a single dose of 1,700 r and may be complete in eighteen hours, regeneration follows rapidly, and a week later many normal follicles may be present. This is true with heavier dosages as well except for a corresponding lengthening of the time interval of response.

The experimental data which we have presented suggest that radiation reaction in the lung need not be a hazard in therapy provided the radiologist realizes the potential danger and is watchful for signs or reaction.

SUMMARY

The lung reacts to radiation as do superficial tissues, with congestion, edema and degenerative changes of cells and intercellular substance. Certain phases of the reaction in the lung are distinct from other forms of pulmonary inflammation. These early alterations we have followed in a variety of laboratory animals as well as in man. The earliest phases of reaction are reversible, producing no permanent change. Our observations, in addition to confirming those recorded in the literature, have added knowledge of the epithelial changes and hyaline membrane and have presented criteria permitting an exact histologic diagnosis for animals and man.

Extensive fibrosis and pleural adhesions may be ascribed to inflammation or infection, intercurrent or resulting from the radiation-induced tissue changes.

Our pathologic data provide a basis for the explanation of the symptoms and roentgenologic pictures in radiation pneumonitis in man.

HISTOLOGIC CHANGES IN THE PITUITARIES OF PARABIOTIC RATS

ISOLDE T. ZECKWER, M.D.

PHILADELPHIA

The pituitary has been shown to be very labile in its cellular pattern and in its secretory response to alteration in the quantity of any of the hormones produced by the peripherally located endocrine organs. These changes in the pituitary have been particularly well studied after ablation of the gonads and after injection of "sex hormones." When any of the various pharmacologic substances that are effective as estrogens and androgens is injected into an animal, the effect is obviously not a continuous, but an intermittent one; the dosage may be far beyond what could occur as a naturally produced secretion; and the substance injected is usually not identical with the corresponding normal product of the gonads.

Parabiosis between a gonadectomized rat and a normal partner, on the other hand, offers a means of studying the effect of continuous hypersecretion by the pituitary and gonads and limits the doses to the amounts that can be naturally produced by these endocrine glands.

It has been frequently demonstrated that the pituitary of a male or a female gonadectomized rat united in parabiosis to a normal or a hypophysectomized female rat hypersecretes gonadotropic hormone which passes over to the partner and stimulates its ovaries, the follicle-stimulating effect predominating and the luteinizing effect being negligible. The rat remains in constant estrus without antigonadotropic effects developing (Du Shane and co-workers¹). The estrogen secreted, however, does not pass back to the castrated rat.

In a normal male rat united in parabiosis to a gonadectomized male or female there is great enlargement of the seminal vesicles and prostate, indicating excessive secretion of androgenic substance by the interstitial cells, but there is no stimulation of seminiferous tubules (Zeckwer²).

There is a voluminous literature on the histologic changes in the pituitary resulting from the injection of estrogens and androgens. In brief, these changes following the administration of estrogens consist of

From the Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia.

1. Du Shane, G. P.; Levine, W. T.; Pfeiffer, C. A., and Witschi, E.: *Proc. Soc. Exper. Biol. & Med.* **33**:339, 1935.

2. Zeckwer, I. T.: *Am. J. Physiol.* **128**:169, 1939.

degranulation of basophils and, with large doses, degranulation of acidophils and hyperplasia of chromophobes. The present experiments gave an opportunity to determine whether naturally produced estrogen and androgen, secreted by rats united to gonadectomized partners, would result in similar histologic changes in the pituitary. It was thought to be of interest to carry out additional experiments based on the same principle, uniting thyroidectomized rats with normal rats, and thyroidectomized rats with gonadectomized rats.

METHODS

White rats were used from a colony maintained in this laboratory for five years. They were fed on Purina. Parabiosis was carried out in the usual way, by suturing together the muscles of the lateral abdominal walls. In one group of experiments 1 rat of a pair was gonadectomized and designated, for convenience, the donor, while the partner was considered the recipient. In other experiments 1 rat of a pair was thyroidectomized instead of castrated, and in a few experiments thyroidectomized rats were united with castrated rats. Controls consisted of normal litter mates of the recipients united to normal litter mates of the donors. After suitable intervals of time the rats were killed with chloroform and their pituitaries immediately fixed in Helly's fluid. Histologic sections of a group of test and control pituitaries were mounted on the same slide and stained simultaneously with Mallory's connective tissue stain. Histologic observations were recorded prior to identifying the slides.

RESULTS

Females United to Gonadectomized Rats.—Twelve females were united in parabiosis with gonadectomized rats (with 7 ovariectomized and 5 castrated males) for periods varying from twenty-one to forty-nine days. Basophils in the pituitaries showed a variable amount of degranulation, some degranulation being present at even the shortest interval of time. Some degranulation of acidophils occurred in 4 rats. In 2 animals this degranulation of acidophils occurred in rats united with ovariectomized females and did not occur in litter mates united with castrated males. In additional rats maintained for a number of months, whose pituitaries could not be sectioned, there was marked stunting of growth of the intact partner and often spontaneous death of this rat. One rat maintained a year showed almost complete degranulation of acidophils and basophils.

Males United to Gonadectomized Rats.—Ten males were united in parabiosis with gonadectomized rats (with 3 ovariectomized females and 7 castrated males) for periods varying from twenty-one to sixty-one days. A slight degranulation of basophils was observed in only 3 rats. Acidophils showed no conspicuous change even in an additional rat maintained one year.

Rats United with Thyroidectomized Partners.—Eight rats (3 males and 5 females) were united to thyroidectomized rats of the same sex for

periods of eighteen to forty-seven days. No conspicuous changes were noted in the pituitaries of the intact rats, and neither their thyroids nor their gonads showed increase in size.

Thyroidectomized Rats United with Gonadectomized Rats.—One thyroidectomized female and 3 thyroidectomized males were united to gonadectomized rats of the same sex. The thyroidectomized male, at thirty-one, forty-seven and sixty-one day intervals, showed the characteristic "thyroidectomy cells" in their pituitaries which would be expected in single thyroidectomized rats. The female, maintained one hundred and eighty-nine days after parabiosis, had a very large pituitary, almost completely degranulated basophils and acidophils, and hyperplasia of chromophobes, an effect which one would expect after prolonged action of an estrogen, whether this was acting on the pituitary of a normal or of a thyroidectomized rat.

Gonadectomized Rats United with Normal or Thyroidectomized Rats.—The pituitaries of the gonadectomized rats united with other rats in the preceding groups showed the typical castration changes unaltered by parabiosis.

Controls.—The controls, consisting of 5 males and 9 females united to normal rats of the same sex, showed no alterations in their pituitaries caused by parabiosis.

COMMENT

The changes in the pituitary resulting from naturally produced hypersecretion of ovaries and testes are the same as those described after the injection of pharmacologic substances effective as androgens and estrogens. The pituitary of the female seems to show more sensitive response than that of the male, and hypersecretion by the ovary affects the pituitary more readily than hypersecretion by the interstitial cells of the testes.

The retardation in body growth of intact rats united with gonadectomized rats over long periods suggests that degranulation of acidophils is accompanied by decreased production of growth hormone, just as Zondek³ accounted for the dwarfing of single rats by large doses of estrogen as being caused by a marked hypophysial deficiency. However, according to the opinion of Severinghaus⁴ in relation to gonadotropic hormones, "the marked degranulation of the basophils and acidophils in the hypophyses of estrogen-treated animals is cytological confirmation of secretion release," and, because there is also hypertrophy of mitochondria and of the Golgi apparatus, he stated that the "anterior lobe of estrogen-treated animals must be regarded as highly active in the elabo-

3. Zondek, B.: *Lancet* 1:10, 1936; 2:842, 1936.

4. Severinghaus, A. E., in Allen, E.; Danforth, C. H., and Doisy, E. A.: *Sex and Internal Secretions*, ed. 2, Baltimore, Williams & Wilkins Company, 1939, p. 1064.

ration as well as release of its secretory products." This concept does not seem to be supported by the present experiments. In fact, in long-continued experiments the intact recipient rat often died spontaneously and before death suggested the picture of pituitary cachexia, a phenomenon to be studied more completely in future experiments.

In single castrated rats the signet ring castration cells are sometimes interpreted as evidence of excessive storage of gonadotropic secretion when there is no end organ for utilization of the secretion. In the present experiments the partner's gonads obviously utilized to the maximum the excessive gonadotropic hormone, and yet castration cells were present as usual in the castrate rat's pituitary. This suggests that a different interpretation should be placed on the significance of the signet ring cells.

SUMMARY

The pituitaries of female rats united in parabiosis with ovariectomized rats showed degranulation of basophils and, in some instances, moderate degranulation of acidophils during the intervals of time studied. Extreme degranulation occurred after a year.

The pituitaries of male rats united in parabiosis with gonadectomized rats showed only occasional very slight degranulation of basophils.

The pituitaries of rats united in parabiosis with thyroidectomized rats showed no conspicuous changes.

After gonadectomized rats were united in parabiosis with thyroidectomized rats, castration cells were found similar to those occurring in single rats. Thyroidectomy cells persisted except in the case of 1 female, maintained for one hundred and eighty-nine days, which showed the usual effects of degranulation by estrogen in very large doses.